

**BIOGAS PRODUCTION FROM
AGRICULTURAL WASTES AND
FEASIBILITY STUDY TO ENRICH
BIOGAS MANURES**

YERASI KAVYA

B.Sc. (Ag.)

**MASTER OF SCIENCE IN AGRICULTURE
(AGRICULTURAL MICROBIOLOGY)**



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BY

YERASI KAVYA

B.Sc. (Ag.)

**THESIS SUBMITTED TO THE ACHARYA N. G. RANGA
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CHAIRPERSON: DR. A. VIJAYA GOPAL



**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY AND
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2014

CERTIFICATE

Ms. Y.KAVYA has satisfactorily prosecuted the course of research and that the thesis entitled **“BIOGAS PRODUCTION FROM AGRICULTURAL WASTES AND FEASIBILITY STUDY TO ENRICH BIOGAS MANURE”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

Date:

Place: Hyderabad

(Dr. A. VIJAYA GOPAL)

Chairman of the Advisory Committee

CERTIFICATE

This is to certify that the thesis entitled **“BIOGAS PRODUCTION FROM AGRICULTURAL WASTES AND FEASIBILITY STUDY TO ENRICH BIOGAS MANURE”** submitted in partial fulfillment of the requirements for the degree of **“MASTER OF SCIENCE IN AGRICUTLURE”** of the **Acharya N. G. Ranga Agricultural University, Hyderabad**, is a record of the bonafide research work carried out by **Ms. Y. KAVYA** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

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Place: Hyderabad

Date

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LIST OF SYMBOLS AND ABBREVIATIONS

Cm	:	Centimetre
&	:	And
M	:	Metre
G	:	Gram
Kg	:	Kilogram
T	:	Tonne
@	:	At the rate of
SEm	:	Standard error of mean
CD (P=0.05)	:	Critical difference at 5 per cent level
<i>et al.</i>	:	And others
%	:	Per cent
NS	:	Non significant
°C	:	Degree Celsius
N	:	Nitrogen
P	:	Phosphorus
K	:	Potassium
OC	:	Organic carbon
pH	:	Pussancea hydrogen
μ	:	Microns
EC	:	Electrical conductivity
CM	:	Conductivity Metre
viz.,	:	Namely
Max	:	Maximum
Min	:	Minimum
FYM	:	Farmyard manure
No.	:	Number
vis-à-vis	:	In relation to
F- test	:	Fisher's test
CFU	:	Colony forming unit
Spp.	:	Species
KB	:	King'B medium

CRD	:	Completely randomized design
RBD	:	Randomized block design
R ₁	:	Replication 1
R ₂	:	Replication 2
R ₃	:	Replication 3
VC	:	Vermicompost
CD	:	Cow dung
Mg	:	Milligram
TKN	:	Total kjeldahl nitrogen
MSW	:	Municipal solid wastes
AZ	:	<i>Azotobacter</i>
TS	:	Total solids
TVS	:	Total Volatile solids
VFA	:	Volatile Fatty Acids
BOD	:	Biological Oxygen Demand
D.O	:	Dissolved Oxygen
D.O ₁	:	Dissolved Oxygen on the first day
D.O ₅	:	Dissolved Oxygen Demand on the fifth day
COD	:	Chemical Oxygen Demand
YEMA	:	Yeast Extract Mannitol Agar
M	:	Cattle Manure
R	:	Rumen fluid
W	:	Tap Water
m ³ .d	:	Deci meter cube
G-R	:	Grass Rabbit
F-G	:	Field Grass
m ³ /m	:	Cubic meter per meter
ppm	:	Parts per million
g/L	:	Gram per litre
mL	:	Milli Litre
CH ₄	:	Methane
L	:	Litre
mmol dm ⁻¹	:	Milli moles per deci meter

TMS	:	Total Mass of Slurry
CH ₄ /g VS	:	Methane per gram volatile solids
ml/g-VS	:	Milli gram per litre Volatile solids
Nm ³ /kgVS	:	Normal cubic metre per kg volatile solids
l/g vs	:	Litre per gram volatile solids
VS/L	:	Volatile solids per litre
g	:	Grams
g Kg ⁻¹	:	gram per kilogram
ms cm ⁻¹	:	Milli siemen per metre
dsm ⁻¹	:	Deci siemen per metre
mg l ⁻¹	:	Milli gram per litre
m ³	:	Cubic metre
m ³ per day	:	Cubic metre per day
m ³ kg ⁻¹	:	Cubic metre per kilogram
g l ⁻¹	:	Gram per litre
mg Kg ⁻¹	:	Milli gram per kilo gram
BD	:	Buffalo Dung
PL	:	Poultry Litter
SW	:	Swine Wastes
PM	:	Poultry Manure
PC	:	Phospho Compost
OKW	:	Organic Kitchen Wastes
DOM	:	Dry matter and organic matter
cm ³	:	Centimetre cube
MWh	:	Million watts per hour
PSB	:	Phosphate Solubilising Bacteria
BSS	:	Biogas Spent Slurry
NaOH	:	Sodium Hydroxide
HCL	:	Hydrochloric acid
H ₂ SO ₄	:	Sulphuric acid
FAO	:	Food and Agricultural Organisation
CIPHET	:	Central Institute of Post-Harvest Engineering and Technology
CO ₂	:	Carbondioxide
CO	:	Carbonmonoxide

H ₂ S	:	Hydrogen sulphide
w/w	:	Weight / weight
h	:	Hours
min	:	Minutes
N	:	Normality
FID	:	Flame Ionization Detector
APHA	:	American Public Health Association.
nm	:	Nanometer
mg ml ⁻¹	:	Milligram per millilitre
K ₂ Cr ₂ O ₇	:	Potassium dichromate
Rs	:	Rupees

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ABSTRACT

The experiment was conducted during 2013-14 at Department of Agricultural Microbiology and Bioenergy, and Department of Soil Science & Agricultural Chemistry, College of Agriculture, Rajendranagar, ANGRAU, Hyderabad.

Cow dung along with other agricultural wastes (press mud, poultry litter, kitchen wastes, maize stalks and fruit wastes) were used for the biogas production in lab scale. Along with the estimation of biogas production different parameters like Total Solids (TS) per cent, Total Volatile Solids (TVS) per cent, Volatile Fatty Acids (VFA), pH, Nitrogen (N), Phosphorous (P), Potassium (K), Organic carbon per cent, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Electrical Conductivity (EC) and methane percentage was estimated.

For each treatment 750 g of substrate and 1500 ml of water was used as inoculum mixture in 3 liters glass bottles. In T₁ 250 g cow dung + 500 g press mud + 1500 ml water, T₂ 250 g cow dung + 500g poultry litter + 1500 ml water, T₃ 250 g cow dung + 500 g kitchen wastes + 1500 ml water, T₄ 250 g cow dung + 500 g maize stalks + 1500 ml water, T₅ 250 g cow dung + 500 g fruit wastes + 1500 ml water and T₆ 750 g cow dung + 1500 ml water were used. The experiment was run for ten weeks and all the parameters were observed in the initial, 7th, 14th, 21st, 28th, 42nd, 56th day of gas production.

At the end of tenth week the gas production was significantly more in T₁ (Cow dung + Press mud) 9903.31 ml, compared to T₆ (Cow dung alone) 8103.31ml, T₂ (Cow dung + Poultry litter) 6079.98 ml, T₃ (Cow dung + Kitchen waste) 4066.63 ml, T₅ (Cow dung + Fruit waste) 3373.32 ml and less in T₄ (Cow dung + Maize stalks) 3099.97 ml.

By the end of the process the degradation of total solids (TS) per cent was significantly more in T₆ (Cow dung alone) 57.16 per cent and less in T₅ (Cow dung + Fruit waste) 32.55 per cent. The degradation of total volatile solids (TVS) per cent was significantly more in T₆ (Cow dung alone) 37.41 per cent and less in T₅ (Cow dung + Fruit waste) 14.32 per cent.

pH in the substrate was estimated and it was significantly more in T₁ (Cow dung + Press mud) 7.66 and less in T₃ (Cow dung + Kitchen waste) 5.77. By the end of the process pH was more in T₆ (Cow dung alone) 6.82 and less in T₅ (Cow dung + Fruit waste) 3.67. The N per cent and P per cent in the process of anaerobic digestion was reduced while K per cent was increased. At the end of the process the reduction in BOD was observed and significantly more reduction was observed in T₅ (Cow dung + Fruit

waste) 58.74 per cent and less reduction in T₄ (Cow dung + Maize stalks) 27.68 per cent. At the end of the process, decrease in COD was observed and was more in T₆ (Cow dung alone) 52.15 per cent and less reduction in T₄ (Cow dung + Maize stalks) 18.77 per cent.

The second experiment on feasibility to enrich the biogas manures with beneficial microorganisms was carried at Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar, ANGRAU, Hyderabad.

The slurry samples from all the six treatments and three replications were collected dried in trays under the sun until the moisture was 50 per cent. The dried manure samples were divided into two and one part was sterilized and the other was used as the same i.e. unsterilized. The population of beneficial microorganisms in the dried manures was estimated and the population of *Pseudomonas* (46.0×10^3 CFU g⁻¹) and *Azospirillum* (24.0×10^3 CFU g⁻¹) was more in T₆ (Cow dung alone), the population of *Rhizobium* was more in T₁ (Cow dung + press mud) 46.0×10^3 CFU g⁻¹ and the population of *Azotobacter* was more in T₂ (Cow dung + Poultry litter) and T₆ (Cow dung alone) 42.0×10^3 CFU g⁻¹.

The beneficial microorganisms used in the study for enrichment were *Rhizobium*, *Pseudomonas*, *Azotobacter* and *Azospirillum*. The organisms were inoculated individually and also as consortia in to the manure samples of all the six treatments and the viability was monitored.

In the unsterilized manure samples enriched individually with beneficial microorganisms the viability of *Rhizobium* was significantly more in T₅ (Cow dung + Fruit waste), for *Pseudomonas* viability was significantly more in T₃ (Cow dung + Kitchen waste), for *Azotobacter* viability was significantly more in T₆ (Cow dung alone) and for *Azospirillum* viability was significantly more in T₅ (Cow dung + Fruit waste). In the sterilized manure samples the viability of *Rhizobium* was significantly more in T₃ (Cow dung + Kitchen waste), for *Pseudomonas* viability was significantly more in T₃ (Cow dung + Kitchen waste), for *Azotobacter* viability was significantly more in T₂ (Cow dung + Poultry litter) and for *Azospirillum* viability was significantly more in T₁ (Cow dung + Press mud).

The viability appears to be good upto the end of fourth week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium*, *Pseudomonas* and *Azospirillum* whereas for *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manures.

In the unsterilized manure samples enriched with consortia of beneficial microorganisms the viability of *Rhizobium* was significantly more in T₅ (Cow dung + Fruit waste), for *Pseudomonas* the viability was significantly more in T₅ (Cow dung + Fruit waste), for *Azotobacter* viability was significantly more in T₆ (Cow dung alone) and for *Azospirillum* viability was significantly more in T₆ (Cow dung alone). In the sterilized manure samples the viability of *Rhizobium* was significantly more in T₁ (Cow dung + Press mud) for *Pseudomonas* viability was significantly more in T₅ (Cow dung + Fruit waste), for *Azotobacter* viability was significantly more in T₁ (Cow dung + Press mud) and for *Azospirillum* viability was significantly more in T₂ (Cow dung + Poultry litter).

The viability appears to be good upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium* and *Azospirillum* whereas for *Pseudomonas*, the viability was good upto the end of fourth week irrespective of treatments in unsterilized biogas manures and upto the end of third week in sterilized manure samples. For *Azotobacter*, the viability appears to be good only upto the end of third week irrespective of treatments in unsterilized biogas manures and upto the end of fourth week in sterilized biogas manures.

CHAPTER I

INTRODUCTION

Energy is one of the most important factor to global prosperity. In today's energy demanding lifestyle, the need for exploring and exploiting new sources of energy which are renewable, sustainable as well as eco-friendly is inevitable. The over dependence on fossil fuels as primary energy source has led to global climate change, environmental pollution and degradation, thus leading to human health problems (Okkerse *et al.* 1999).

Worldwide energy crisis directed the attention to the alternative sources of energy instead of underground fossil fuel. The possible shortage in fossil fuels and environmental problems that the world is facing today requires long-term potential actions for sustainable development. In this regard, renewable energy resources appear to be one of the most efficient and effective solutions (Kaygusuz *et al.* 2002). Biogas has globally remained a renewable energy source derived from plants that use solar energy during the process of photosynthesis. Biogas is a readily available energy resource that significantly reduces greenhouse-gas emission compared to the emission of landfill gas to the atmosphere (Nabuuna and Okure, 2005). Being a source of renewable natural gas, it has been adopted as one of the best alternatives for fossil fuels after 1970's world energy crisis (Imam *et al.* 2013).

Currently, as the fossil-based fuels become scarce and more expensive, the economics of biogas production is turning out to be more favourable. Biogas originates from bacteria in the process of biodegradation of organic material under anaerobic conditions. Anaerobic digestion, a biological conversion process that occurs in the absence of oxygen, has a number of advantages for waste conversion and ultimately producing methane and carbon dioxide (Garba and Sambo, 1995). It consists of a varying proportion of CH₄ (methane) and CO₂ (carbon dioxide) and traces of H₂S, N, CO and water vapour. Methane is the most valuable component under the aspect of using biogas as a fuel, the other components do not contribute to the calorific value and are often "washed out" in purification plants in order to obtain a gas with almost 100 per cent CH₄. Methane is the flammable compound in biogas. Biogas production in India was 45.74 lakhs m³ per day and in Andhra Pradesh it was 4.28 lakhs m³ per day as on 31.03.2012.

Biogas is smokeless domestic fuel which reduces the incidence of eye and lung diseases among the users while reducing the burden on forest, reduction in drudgery of

women and children. It is a better and cheaper fuel for cooking, lighting and power generation. The use of agricultural wastes for biogas generation offers several benefits such as the production of safe, clean energy resource that can be stored and used more efficiently. The production of stabilized residue that retains the fertilizer value of original material which has superior nutrient qualities over the usual organic fertilizer. Indirect benefits of biogas generation include the potential for partial sterilization of wastes with consequent reduction of the public health hazard of faecal pathogens and reduction of fungal and other plant pathogens from crop residue (Sagagi *et al.* 2009).

Recently, large volume of cow dung generated from feedlot farming increased annually, most of which are disposed into landfills or applied to the land without treatment. Anaerobic digestion provides an alternative option for energy recovery and waste treatment. Handling of wastes and their management is a problem now, so one approach to utilize the waste is to use it as energy resource. Moreover, (Pound *et al.* 1981) it is observed that biogas production units provide a decentralized fuel supply and waste management system both of which are very attractive particularly in rural areas of developing countries.

Press mud is a solid residue, obtained from sugarcane juice before crystallization of sugar. Generally press mud is used as a manure in India. It is a soft, spongy, light weight, amorphous, dark brown to black coloured material. It generally contains 60-85 per cent moisture (w/w); the chemical composition depends on cane variety, soil condition, nutrients applied in the field, process of clarification adopted and other environmental factors (Agrawal *et al.* 2011). Press mud from sugar factory typically contains 71 per cent moisture, 9 per cent ash and 20 per cent volatile solids, with 74-75 per cent organic matter on solids. Sugar molasses has methane potential (*i.e.* CH₄ per ton of raw material) of 230 m³.

The present methods for disposal of press mud are not economically suitable and pollute the environment. As it contains appreciable proportion of biodegradable organic matter, it has very good potential for the production of biogas. The press mud contains volatile solids, lignin, lipids, cellulose, hemicelluloses etc which favours biogas production. It also has good proportion of nitrogen. This makes it a very good material for generation of bioenergy (methane) by anaerobic biomethanation. The advantage of using press mud is that the sludge coming out from the digester is a good fertilizer and press mud can be used in combination with other raw materials to increase the efficiency (Agrawal *et al.* 2011).

Maize is a cereal crop that is grown widely throughout the world. The wastes of maize which are left behind after harvest include the husks, chaff, stalks and the leaves (Oseni and Ekperigin, 2007). Chaffs, cobs and stalks are among the prominent wastes associated with maize. The maize wastes just like other agro-based wastes are indiscriminately left on the farm to be mineralized and used by other crops. Anaerobic digestion of these wastes will not only produce biogas and a residue organic waste that has superior nutrient qualities over the usual organic fertilizer, it also minimizes environmental pollution as it reduces greenhouse emission (Voss, 2007).

India stands second in the production of Fruits and Vegetables in the world. It contributes about 10 and 14 per cent of Fruit and Vegetable in the world production (Goutam *et al.* 2007). Vegetable Wastes are created during harvesting, transportation, storage, marketing and processing. Due to their nature and composition, they deteriorate easily and cause foul smell. According to Food and Agricultural Organization (FAO), the estimated Fruit and Vegetable wastes percentage for each commodity group in each step of the food supply chain are 15, 9, 25, 10 and 7 per cent in agricultural production, post harvest handling and storage, processing and packaging, distribution and consumption respectively in South and South-East Asia (FAO 2011). 18 per cent of India's fruit and vegetable production valued at Rs. 13,300 crores is wasted annually, according to data from the Central Institute of Post-Harvest Engineering and Technology (CIPHET), November 2013. The high moisture and organic content in these wastes can be utilized in biological treatment like anaerobic digestion.

It is inevitable that with large volume and high density poultry productions, there will be large quantities of poultry waste produced. Poultry droppings are available in India to the extent of 3.6 M.t per annum. These wastes used as feed material to produce biogas can tap additional energy from the otherwise wasted energy and make the poultry industry co-exist with the environment and improve sanitation and reduce environmental pollution (Singh *et al.* 2008).

Organic manures in agriculture add much needed organic matter and minerals to the soil. The important manures used in organic farming are compost, vermicompost, biogas spent slurry, green manures and liquid organic manures like panchagavya, jeevamruth, beejamruth etc. The beneficial effects of organic manures in agricultural production and soil fertility are known from many decades, but they are inadequate in nutrient supply and low in nutrient concentrations. The total nutrients recycled from organic matter decomposition are much less than the amount of nutrients utilized by the crop plants. This necessitates the enrichment of manures with beneficial microbial

inoculants like free living nitrogen fixers, phosphate solubilizers *etc.* to improve the nutritional status of the manures. The enrichment of manures with beneficial microbial cultures result not only in improvement of nutritive value but also in higher growth and yield of crops. The microbial enrichment of organic manures will further contribute to the enhancement of phosphate solubilisation and nitrogen fixation (Hema *et al.* 2012). Hence, the present investigation was also aimed at enrichment of biogas manure with beneficial micro organisms.

The present work was planned to study the use of these agricultural wastes in combination with cow dung for biogas production and an attempt has been made to analyze the microbial status of biogas slurry before and after their microbial enrichment.

Objectives of investigation:

- 1) To optimize the methane gas evolution from agricultural wastes with cow dung on a laboratory scale study by anaerobic digestion.
- 2) To evaluate the biogas manure for the viability of beneficial microorganisms.

Chapter II

REVIEW OF LITRATURE

The demand of energy has been increased over the years as the sequence by increasing of the world population. Currently, the energy consumption was rising in relation to economic growth. In this situation, with the abundant and varieties of biomass and agricultural wastes should have the great challenge and opportunity for the supply to anaerobic digestion for biogas production. The present investigation is taken up to study of methane gas production by utilizing different agricultural wastes.

This chapter reviews the work done on the biogas production using different agricultural wastes and the enrichment of the biogas manures with beneficial micro organisms.

2.1. OPTIMIZATION OF METHANE GAS EVOLUTION FROM AGRICULTURAL WASTES WITH COW DUNG IN A LABORATORY SCALE STUDY BY ANAEROBIC DIGESTION.

2.1.1 Total solids (TS) and total volatile solids (TVS)

Singh *et al.* (2012) explained anaerobic digestion as a biological process which involves breakdown of complex substances. The efficiency of digestion system can be evaluated in terms of organics and solid content. The Total solids (TS) and Volatile solids (VS) were of important consideration in loading the reactor. VS per cent was the measure of the degradable components that gives the estimates of methane production. It was stated that VS per cent of cow dung generally lies between 80 per cent and 86 per cent of TS.

Aromsukkho *et al.* (2011) investigated biogas yield using different mixtures of pig manure and vegetable wastes comparing with pig manure or vegetable wastes alone. The biogas production parameters as depending on feed condition such as total solid (TS) and the ratio of feed were considered. The substrates were estimated for the TS per cent and vegetable wastes like Chinese cabbage, Chinese white cabbage and tomatoes were with 4.62 per cent, 8.87 per cent and 4.33 per cent total solids respectively. The 10 digesters were set for monitoring the parameters of 4-8 per cent TS with the ratio of pig manure to vegetable wastes at 100:0, 25:75, 50:50 and 75:25. The highest biogas yield was found at the pig manure to vegetable wastes ratio of 25:75 and 8 per cent TS with highest biogas production of 38.49 L. The maximum capacity of biogas yield was after 8 days of digestion.

Patil *et al.* (2011) explained water hyacinth as a very good biogas producer and needs minimal pre-treatment (drying and grinding) to enhance the biogas yield. The anaerobic co-digestion of water hyacinth with poultry litter and water hyacinth with cow dung in different ratios were carried out in 250 ml batch digesters for a 60 days retention period. Co-digestion was carried out in mesophilic temperature range of 30 to 37°C with a total solid concentration of 8 per cent in each sample (fermentation slurry). The biogas was collected by the downward displacement of water, and was subsequently measured. The result of the study has shown that anaerobic co-digestion of dried and ground water hyacinth with poultry litter had the highest cumulative biogas yield.

Tumutegyreize *et al.* (2011) showed the peels from different banana cultivars yielded methane in the same range as long as the particle size was the same. Particle size has a significant effect on the quality, but not quantity of methane yield. The projected optimal particle size of *matooke* peels for anaerobic digestion was 6.73 mm.

Budiyono *et al.* (2010) conducted a series of laboratory experiments using 400 ml biodigester were performed in batch operation mode. 100 grams of fresh cattle manure (M) was fed to each biodigester and mixed with rumen fluid (R) and tap water (W) in several ratio resulting six different M:W:R ratio contents i.e. 1:1:0; 1:0.75:0.25; 1:0.5:0.5; 1:0.25:0.75; and 1:0:1 (correspond to 0; 12.5; 25, 37.5; 50, and 100 per cent rumen, respectively) and six different total solid (TS) contents i.e. 2.6, 4.6, 6.2, 7.4, 9.2, 12.3, and 18.4 per cent. The results showed that the rumen fluid inocula caused biogas production rate and efficiency increase more than two times in comparison to manure substrate without rumen fluid inocula. The best performance for biogas production was the digester with rumen fluid and TS content in the range of 25-50 per cent and 7.4-9.2 per cent, respectively.

Ofoefule *et al.* (2010) carried anaerobic digestion of some animal dung like cow, swine, rabbit, poultry and goat. The TS per cent in the animal dung was 36.38 per cent, 27.01 per cent, 15.65 per cent, 17.02 per cent and 19.89 per cent respectively and the TVS per cent in the animal dung was 77.38 per cent, 72.94 per cent, 82.35 per cent, 83.80 per cent, 68.80 per cent respectively. The average volume of bio gas produced was 44, 40, 37, 33 and 31 dm³ per total mass of slurry respectively. The total volume of biogas produced by each dung for the whole period was compared and cow dung produced highest volume of biogas while goat dung produced least volume.

Rouf *et al.* (2010) worked on the optimization of biogas generation from press mud using batch reactor. Press mud, cow dung and kitchen wastes contain 22.2 per cent,

24.9 per cent and 11.4 per cent TS and 77.1 per cent, 77.2 per cent and 92.0 per cent TVS respectively.

Agrawal *et al.* (2011) explained that the press mud contains 77 per cent volatile solids, lignin, lipids, cellulose, hemicelluloses etc which favours biogas production. It also has good proportion of nitrogen. This makes it a very good material for generation of bioenergy (methane) by anaerobic bio methanation.

Uzodinma and Ofoefule (2009) carried an investigation on the production of biogas, a low natural gas, from equal blending of field grass with some animal wastes which include cow dung, poultry dung, swine dung and rabbit dung. The TVS per cent in the substrates was 75.85 per cent, 71.40 per cent, 73.95 per cent, 79.25 per cent and the nitrogen per cent in the substrates was 1.50, 1.92, 1.20, 1.76 respectively. The highest average volume of gas production from grass-rabbit (G-R) blend was 3 times higher than that of F-G alone. Overall results indicate that the biogas yield and onset of gas flammability of field grass can be significantly enhanced when combined with rabbit and cow dung.

Yokoyama *et al.* (2007) conducted experiments using cow dung for biogas production and the composition of cow dung was estimated. Cow dung contains 15 per cent TS, 85 per cent TVS and the pH was 6.6.

Osman *et al.* (2006) studied biogas production from different agricultural wastes. Wheat straw, ground nut shells and sugar cane bagasse were used as substrates in the process. TS per cent in the substrates was 18, 18.2 and 8.85 where as TVS per cent was 85, 93.5 and 86.45 respectively.

Santana *et al.* (1980) explained that there was a linear increase in the gas production between 1 per cent and 8 per cent total solids. The faeces and urine from each pair of bull were collected daily and diluted to 1, 3, 5 and 8 per cent TS. The resulting 8 slurry mixtures were used as substrate in the anaerobic digesters. Biogas production increased linearly with increasing TS concentration. This can be attributed to a greater concentration of organic matter being available for degradation to methane and carbon dioxide. It was suggested that under commercial conditions 8 per cent TS may be nearer the optimum from economic and management points of view.

2.1.2 Total Volatile Fatty acids (VFA)

Pham *et al.* (2013) recommended methods to measure biogas production potential of animal manure. VFA in the cow manure and pig manure was 2.67 and 6.65 g kg⁻¹ respectively.

Siedlecka *et al.* (2008) studied volatile fatty acids (VFAs) are present in environmental waters in the range of 1 to 5,000 ppm and different methods have been reported the distillation method followed by potentiometric titration, spectrophotometric and gas chromatographic methods.

Vedrenne *et al.* (2007) studied the effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. VFA in the cow dung, pig manure and duck manure was estimated as 0.3-7.4, 1.4-14 and 1.8 g l⁻¹ respectively. The methane content in the swine slurries varied from 244 to 343 L CH₄ per kg added, from 204 to 296 L CH₄ per kg VS added for dairy cattle slurries and equalled 386 and 319 L CH₄ kg per VS added respectively for calves and duck slurries.

Lahav *et al.* (2004) explained that an increase in VFA concentration was the first practical measurable indication that an anaerobic treatment system was in stress. The VFA concentration in properly running anaerobic digesters was typically stable and low and ranging between 0.5-2.0 mmoldm⁻¹.

Lahav *et al.* (2002) verified the feasibility and accuracy of a novel on-site method for VFA and alkalinity measurements as means of control of industrial anaerobic reactors. VFA concentration is typically determined with an accuracy of greater than 95 per cent. For concentrations above 200 mg l⁻¹ as HAc typical accuracy is within ± 98 per cent, with very good repetition in results. The results show that when executed properly, the method gives very accurate and repetitive results, using a relatively simple procedure that can be easily implemented in field laboratories. In order to increase the measurement accuracy of samples containing VFA concentrations lower than 100 mg l⁻¹ as HAc, it is recommended to spike the sample with a known concentration of acetic acid.

Sans *et al.* (1993) studied the volatile fatty acids (VFA) production by means of anaerobic fermentation of urban organic wastes. The results showed an important VFA production at mesophilic range of temperature (37°C). Particularly, VFA concentration in the outlet sludge went from 11.8 g l⁻¹ at 2 days of retention time up to 23.1 g l⁻¹ at 6 days of retention time.

2.1.3 pH

Ukpai *et al.* (2013) investigated on the anaerobic digestion in generating biogas from three types of wastes: Cow dung, Cowpea and Cassava peeling. During the digestion period, the volume of biogas production and the changes in pH indicate that at neutral pH, the highest peak of gas production was attained and that at slightly acidic pH range, there was no gas production. The cow dung had the highest cumulative

biogas yield of 124.3 L per total mass of slurry (TMS) while cow pea had 87.5 L per TMS and cassava peeling with lowest cumulative biogas yield of 87.1 L per TMS within the retention period. The result obtained from the gas production showed that cowpea produced the highest methane content of 76.2 per cent, followed by cow dung with 67.9 per cent content and cassava peeling has the least methane content of 51.4 per cent.

Putri *et al.* (2012) indicated the production of biogas from livestock waste manure as one of the alternative ways for utilization of organic wastes. He explained variable A (manure and water) 1:3 ratio and the variable B (manure and rumen) with 1:2 ratio produced the highest volume of biogas compared to other ratios. The highest biogas production occurred on average at day 23. Generally the addition of water and rumen can increase the production of biogas since both raw materials supporting the two important stages in the biogas production (hydrolysis and acetogenesis) in certain level. The degree of acidity was maintained in the range of 6.6 to 7.6 as methanogenic bacteria can only work at that pH range.

Saha *et al.* (2012) clearly explained the relationship between the pH of the slurry used in the biogas production and the volume of biogas generated. At neutral pH (7.0) the volume of biogas produced was 1250 ml where as at acidic pH (5.2) the gas produced was only 300 ml.

Velmurugan and Alwar Ramanujam (2011) demonstrated the anaerobic digestion of vegetable wastes (Banana stem, Cabbage and Ladies finger) in a fed-batch laboratory scale reactor at mesophilic conditions (35⁰C). pH in the range of 6.8 to 7.4 should be maintained in the anaerobic digestion process, which was the optimum range for methanogens growth. The vegetable wastes were fed with an average pH of 5.75 and the reactor residue average pH was 7.55 which was slightly above the optimal pH. The TS per cent in the substrates were estimated as 6.55 per cent, 8.50 per cent and 12.28 per cent in banana stem, cabbage, ladies finger respectively and the total volatile solids per cent in the substrates were estimated as 83.21 per cent, 90.45 per cent and 90.65 per cent. Methane yield was 0.3871 CH₄ g⁻¹ VS added .The average methane content in the biogas was 65 per cent and the methane yield was 0.3871 CH₄ g⁻¹ VS added for the selected types of wastes. Hence he concluded that, it was noteworthy that vegetable wastes were potential source for energy production.

Cioabla and Ionel (2010) mentioned that the quality of the produced biogas was closely related to the type of waste biomass used, but by utilization of the by products (fertilizer or fuel) the economy of the process might turn into a favourable one. The

results showed that the origin and quality of the input raw material used is very important, related with the type of biomass, the duration of the batch fermentation, and also the ration between solid matter and liquid volume. The main parameters of influence for the process of anaerobic fermentation are the temperature regime, the pH of the suspension and the type of biomass and its chemical characteristics.

Espinosa-Solares *et al.* (2010) conducted experiments on the short term effects of temperature changes in a pilot plant for the production of biogas from poultry litter. The chemical composition of the substrate, the effluent and the biogas were monitored throughout the experiments. The pH variations in the poultry litter was in between 7.3 and 7.7. Initially pH was 7.5 in the poultry litter and later it was decreased to 7.3 finally the pH was 7.6. The litter was diluted with fresh water to a concentration of solids of 5-7 per cent. The average influent COD was 52 g l⁻¹ while the ammonia concentration was 17 g l⁻¹ throughout the experiment.

Yadvika *et al.* (2004) stated recirculation of effluent slurry on daily basis and stirring of the digester's contents by using simple techniques for enhancing biogas production seems to be quite viable under rural conditions. Keeping various parameters within the desired range also improves gas production but the practical difficulty lies in maintaining and monitoring these regularly. It is a crucial point which needs due consideration since a slight change in pH or temperature could otherwise result in reduction of gas production. Similarly formation of volatile fatty acids beyond a particular range hinders the methane production. Loading rate and solid concentration should be properly balanced and continuously maintained.

2.1.4 Electrical conductivity (E.C)

Yadav *et al.* (2013) conducted experiments and showed results that indicate cow dung and biogas plant slurry can be used as a raw material in vermicomposting. The substrate cow dung has an E.C of 1.2 to 2.2 dsm⁻¹ and biogas plant slurry with an E.C of 1.0 to 1.4 dsm⁻¹. The nutrients in the cow dung like N, P and K were 6.5 to 8.6 g kg⁻¹, 5.0 to 8.7 g kg⁻¹ and 2.8 to 7.8 g kg⁻¹ respectively. While in the biogas plant slurry the N, P and K were 5.2 to 10 g kg⁻¹, 5.0 to 5.8 g kg⁻¹ and 1.3 to 4.2 g kg⁻¹ respectively. The organic carbon content was 425 to 550 g kg⁻¹ for cow dung and 400 to 470 g kg⁻¹ for biogas plant slurry.

Zerrouki *et al.* (2013) worked on the biogas production from the apricot and orange juice cocktail. During experiments various parameters like Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), pH were recorded. The substrates used contain apricot and orange cocktail that comes with pulp and the pH and electrical

conductivity (E.C) of the substrate was found to be 7.39 and 2.06 ms cm⁻¹ respectively. In the case of thermophilic mode a significant decrease of the organic compounds was seen until reaching a value of 200 mg l⁻¹ after 9 days. In the case of mesophilic temperature and under the same conditions the chemical oxygen demand was slightly higher and less than 250 mg l⁻¹. Fifty four to sixty percent chemical oxygen demand removal was reached.

Xing *et al.* (2007) evaluated volatile solids in the layer manure was 19.57 g kg⁻¹ on an average. The electrical conductivity in the layer manure samples was 7.04 ms cm⁻¹ on an average.

2.1.5 Nitrogen (N), Phosphorous (P) and Potassium (K) content of different substrates.

Patil *et al.* (2013) studied the isolation and enrichment of sugar press mud (spm) adapted microorganism for production of biofertilizer by using sugar press mud. The composition of press mud was studied and it contains 0.22 per cent nitrogen, 0.2 per cent phosphorus and 1.27 per cent potassium.

Wani *et al.* (2013) worked on the nutrient content and different physio-chemical parameters in garden waste, kitchen waste and cow dung. He explained the organic carbon content in cow dung (18.4 %) was more compared to the kitchen wastes (13.3 %) and garden wastes (11.7 %). Also explained that the N (1.97 %) and P (0.62 %) content was more in the cow dung compared to kitchen waste and garden wastes, whereas K content more in kitchen wastes (1.01 %) was compared to cow dung (0.88 %) and garden wastes (0.60 %).

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. The studies concluded among the enriched manures, enriched vermicompost had significantly highest microbial load, N, P, K and micronutrient content followed by enriched compost and biogas slurry. Biogas slurry had 1 per cent N, 0.3 per cent P and 0.75 per cent K before enrichment and 1.25 per cent N, 0.5 per cent P and 0.88 per cent K after enrichment.

Yasin and Wasim (2011) concluded that feeding materials and its levels significantly affected the slurry temperature, gas production and pH value. The interaction effect of feeding material and its levels was found significant on slurry temperature and pH value. The highest gas production i.e. approximately 0.92, 0.82 and 0.79 m³ per day was obtained from buffalo dung + poultry litter (BD+PL), BD and BD+ swine wastes (SW), respectively at 40 kg per day feed rate. The average slurry temperature within plant remained between 26 to 31°C, approximately 5-7°C below the

atmospheric temperature. The average slurry temperature of BD, BD+PL and BD+SW treatments remained in the range of 26-28°C, 28-29 °C and 29-31°C, respectively. The average pH values of BD, BD+PL and BD+SW treatments were found to be 6.6, 6.5 and 6.8, respectively. There was an increase in the nitrogen content of every material after fermentation in the plant. This increase was from 1.12 to 1.47 percent for BD, 1.25 to 1.47 percent for BD+PL and 1.37 to 1.54 per cent for BD+SW.

Islam *et al.* (2010) examined the effectiveness of biogas slurry (liquor from anaerobic digestion process) as a nitrogen source for the production of fodder maize (*Zea mays*). They reported that biogas slurry had 5.88 mg kg⁻¹ N, 2.72 mg kg⁻¹ P, 1.33 mg kg⁻¹ K and 0.79 mg kg⁻¹ S.

Kolar *et al.* (2010) tested the procedure of combined phytomass utilization Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB) proposed for ensiled grass matter from the aspect of suitability of its use for a typical substrate of new Czech biogas stations, a mixture of cattle slurry, maize silage and grass haylage. N, P and K analysis of the substrates was carried and concluded that cattle slurry was with more nutrients 2.4 per cent, 1.3 per cent, 5.3 per cent compared to maize silage 0.1 per cent, 0.2 per cent, 1.4 per cent respectively.

Birnin-Yauri *et al.* (2009) worked on the anaerobic fermentation of cattle dung and cattle egret droppings to determine their biogas yielding potentials. The N, P and K analysis on the samples showed greater contents in undigested sample of cattle dung and contrarily, greater contents in digested sample of cattle egret droppings. The N, P and K in cattle dung undigested samples was 1.12 per cent, 0.25 per cent and 10.28 per cent. Whereas the N, P and K in cattle dung egret droppings digested samples was 1.72 per cent, 0.61 per cent, 10.28 per cent respectively.

Singh *et al.* (2008) conducted chemical tests on the poultry slurry and showed that the concentration of potassium was increased and the concentration of phosphorus and nitrogen were decreased on anaerobic digestion. The percentage of N in the raw poultry waste was 2.01. It decreased gradually to 2.00 during the first two weeks of fermentation and then to an average of 1.79 percent finally. The P content of the raw material was reported to be 6.66 percent. It was decreased to 4.37 percent after two weeks digestion and finally 5.85 percent was recorded as the average value. As for K, it increased from 3.45 percent to 4.15 percent after two weeks digestion process with final average value of 5.51 percent.

Ghosh *et al.* (2004) conducted field experiments on deep vertisols of Bhopal, India to evaluate the manurial potential of three organic manures: farmyard manure

(FYM), poultry manure (PM) and phosphocompost (PC). From his experiments he found that poultry manure contains 2.14 per cent N, 1.09 per cent P and 1.3 per cent K.

Ramana *et al.* (1999) studied the nutrient status of press mud and explained that press mud contain 20-30 per cent organic matter, 0.94-1.3 per cent nitrogen, 1.66-2.74 per cent phosphorus and 0.46- 1.3 per cent potassium.

Demont *et al.* (1991) analysed NPK content in biogas slurry and reported that composted slurry had 0.5-1.0 per cent N, 0.5-0.8 per cent P and 0.6-1.5 per cent K. They demonstrated the superior manurial value of composted slurry over ordinary compost.

Gupta (1991) analysed the nutrient status of sun dried biogas slurry and reported that it had 1 per cent N, 0.23 per cent P and 0.84 per cent K.

2.1.6 Organic carbon

Yadav *et al.* (2013) conducted experiments and showed results that indicate cow dung and biogas plant slurry can be used as a raw material in vermicomposting. The substrate cow dung has an E.C of 1.2 to 2.2 dsm^{-1} and biogas plant slurry with an E.C of 1.0 to 1.4 dsm^{-1} . The nutrients in the cow dung like N, P and K were 6.5 to 8.6 g kg^{-1} , 5.0 to 8.7 g kg^{-1} and 2.8 to 7.8 g kg^{-1} respectively. While in the biogas plant slurry the N, P and K were 5.2 to 10 g kg^{-1} , 5.0 to 5.8 g kg^{-1} and 1.3 to 4.2 g kg^{-1} respectively. The organic carbon content was 425 to 550 g kg^{-1} for cow dung and 400 to 470 g kg^{-1} for biogas plant slurry.

Stumpe *et al.* (2012) studied organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. The organic carbon per cent in the biogas slurry was 23.2 per cent.

Suthar (2010) worked on the recycling of agro industrial sludge through vermitechnology. Vermicomposting bedding material was analyzed for its different physic-chemical parameters. The pH of the cow dung was 8.5 and in biogas slurry the pH was 7.3. The organic carbon in the cow dung was 329.8 g kg^{-1} , in biogas slurry it was 359.5 g kg^{-1} and in vegetable wastes was 390.1 g kg^{-1} .

Bernal *et al.* (2009) worked on the composting of animal manures. The pH in the animal manures was determined. The pH in liquid poultry manure was 7.9-8.8 and in solid poultry manure was 7.6. The organic carbon content in liquid poultry manure was 11 – 112 g kg^{-1} and in solid poultry manure was 103 – 597 g kg^{-1} .

Bouallagui *et al.* (2004) explained the psychrophilic, mesophilic and thermophilic process that has been used in the laboratory scale experiments of fruit and vegetable wastes for anaerobic digestion. The TS per cent in the fruit and vegetable

wastes substrate was found to be 11 per cent on an average. The organic carbon content of the substrate was found to be 50.9 per cent on an average.

Jeyabal *et al.* (2001) studied the composition of TS per cent in cow dung, press mud and in the biogas digested slurry. He concluded that total solids per cent was more in the press mud (29 %) compared to that in the cow dung (18.6 %). The composition of nutrients like N, P and K in cow dung, press mud and in the biogas digested slurry was estimated. N (1.65 %) and K (0.81 %) were more in the cow dung than in the press mud while P is more in press mud (1.20 %) compared to cow dung (0.70 %). Organic carbon per cent was more in cow dung (47.3 %) compared to that in the press mud (44 %). Where as in the biogas digested slurry the organic carbon per cent was 27.3 per cent.

2.1.7 Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

Li and jha (2014) studied the characteristics of semi-dry anaerobic digestion of cow dung and pig manure mixture under psychrophilic condition. The chemical oxygen demand (COD) in the substrates was estimated. In cow dung the COD was 150.54 g l⁻¹ and in pig manure 163.63 g l⁻¹.

Deng *et al.* (2013) used swine slurry in different fractions and explained its influence on biogas fermentation. He explained that the high solids content liquid had 2.48–5.42 times greater COD content and 2.81–5.92 times greater BOD₅ than the raw slurry and had a quicker degradation rate according to the kinetics equation. The high solids content liquid, whose volume accounted for 9.72–28.5 per cent of that of the raw slurry, contained 52.7–70.6 per cent of the chemical oxygen demand (COD), 57.6–80.1 per cent of the biological oxygen demand (BOD₅).

Gonzalez *et al.* (2013) carried work on the thermo-chemical pre-treatment to solubilize and improve anaerobic biodegradability of press mud. The characteristics of press mud were studied and the total solids (TS) and the total volatile solids (TVS) were estimated to be 9.09 per cent and 80.84 per cent respectively and the COD was 86.20g l⁻¹.

Owczuk *et al.* (2013) explained that waste products from agricultural industry were a valuable source of feedstock for biogas production. Seven different kinds of feedstock were loaded into the digesters (different swine manure to maize silage ratios). It was found that the cumulative biogas production increased with increasing proportions of the maize silage, however, the methane production was achieved more slowly at higher rather than lower organic loadings. The VS per cent in the swine manure and maize silage was 60.9 per cent and 95.9 per cent respectively. The pH of swine manure and maize silage used was 7.25 and 6.88 respectively. During the

anaerobic digestion the pH variation was in between 7.05 and 7.65. COD of the maize silage was 285 mg O₂ per gram. The highest biogas and methane production was obtained for 1:1 silage to manure ratio, but the kinetics showed long inhibition period where no biogas production occurred. Such effect was undesired in industrial scale, so the 4:1 proportion of silage and manure was more suitable for full-scale fermentation. The obtained biogas characterized high methane content which predispose it as a substrate for biomethane production.

Abubakar *et al.* (2012) investigated the effectiveness of cow dung for biogas production and presented the performance characteristics of the anaerobic digestion in batch and semi-continuous operations. The pH of cow dung was in between 7.1 and 7.4 and during the anaerobic digestion there were variations in between 6.5 to 7.6. Under these conditions, the cow dung digestion reaches 47 per cent volatile solids reduction and 48.5 per cent COD with biogas yield of 0.15L biogas per kg volatile solids added. Despite large variations in pollutant concentrations, an improved performance of anaerobic digestion of the biodegradable fraction of cow dung was achieved. COD removal efficiency was comparatively lower than the commonly obtained COD removal from cattle manure (51-79 %). The results showed that cow dung might be one of the feedstock for efficient biogas production and waste treatment.

Salunkhe *et al.* (2012) clearly explained about kitchen solid waste i.e. food waste based biogas system was more useful than conventional i.e. cow dung based biogas system. Slurry generated in kitchen solid waste based biogas digester contains less BOD as compared to conventional biogas plant. BOD of the kitchen solid waste slurry was 18 mg l⁻¹ where as for cow dung slurry 30mg l⁻¹. Nutrients like NPK in wet slurry of kitchen wastes were 1.96, 0.96 and 3.32 respectively and NPK in dry slurry were 0.98, 0.84 and 1.37 respectively. But NPK in cow dung were 0.56, 0.35 and 0.78 respectively. NPK in slurry were four times more than that of cow dung.

Bhumesh Singh and Sai (2011) studied on biogas generation from dairy effluent and control of water pollution has been viewed with the aim of control of water pollution through treatment of dairy waste as well as generation of biogas. Environmental parameters like Temperature, pH, Biological Oxygen Demand (BOD) & Chemical Oxygen Demand (COD) was taken in to account. No change in the average value of temperature and pH was recorded but BOD and COD reduced to the extent of 50 per cent. All parameters however showed statistically significant differences at 5 per cent level between inlet and outlet point. Gas generation fluctuated between 5 m³ per day to maximum 4.5 m³ per day with an average of 3m³ per day was recorded.

2.1.8 Bio gas production and methane percentage

Aragaw *et al.* (2013) suggested the need of anaerobic co-digestion strategies to enhance biogas production when treating certain residues such as cattle or pig manure. Co-digestion of food waste with animal manure or other feedstocks with low carbon content can improve process stability and methane production. In this study, anaerobic digestion and co-digestion of cattle manure with organic kitchen waste using rumen fluid as inocula had been experimentally tested to determine the biogas potential. Co-digestion substantially increased the biogas yields by 24 to 47 per cent over the control (organic kitchen waste and dairy manure only). The highest methane yield of 14,653.5 ml per g VS was obtained with 75 per cent Organic Kitchen Waste (OKW) and 25 per cent cattle manure (CM) additions. In contrast, addition of 75 per cent cattle manure caused inhibition of the anaerobic digestion process, and its cumulative methane yield was 23 per cent lower than that with 25 per cent cattle manure addition

Aremu and Agarry (2013) clearly showed that poultry droppings could serve as a suitable substrate for biogas production. The study was carried out through anaerobic fermentation using poultry droppings as main substrate and corn-cob and waste papers as co-substrate. The fermentation was carried out at temperatures between 27 – 35 °C and pH range of 4.2-8.0 for a period of 30 days. The initial pH of the poultry droppings was 7.2, for poultry droppings + untreated co-substrates 6.8 and for poultry droppings + treated co-substrates 7.5 were as at the end on 30th day the pH was 4.8, 4.9 and 6.9 respectively.

Chamarthi *et al.* (2013) explained that the temperature plays a crucial role in fermentation of the slurry for the better action of methanogens. An increased temperature in the digester tank facilitates faster reaction rates and hence faster gas yields are produced. The gas samples which are collected through gas balloons from the biogas plant were taken to the laboratory for determination of composition of biogas by using gas chromatography. The experimental results shown that the methane yield composition increased from 63.40 per cent to 64.22 per cent in the transparent dome digester plant when compared to that of mild steel dome digester.

Imam *et al.* (2013) showed that flammable biogas can be produced from cow dung and poultry litter through anaerobic digestion for biogas generation. The total gas production from 13.5kg of cow dung is 0.46 m³. This means that 0.034 m³ kg⁻¹ of biogas is produced from cow dung. The total gas production from poultry waste is almost twice (0.79 m³) to that of cow dung. This gives 0.058 m³ kg⁻¹ of biogas production from poultry waste.

Prabhudessai *et al.* (2013) worked on anaerobic digestion of locally available agro wastes like coconut oil cake, cashew apple waste, and grass from lawn cuttings. The most productive agro waste, in terms of methane yield, was coconut oil cake and grass. The results showed that the initial volatile solids concentration significantly affected the biogas production. The methane yield from coconut oil cake was found to be 383ml CH₄ per g VS and 277 ml CH₄ per g VS added at 4 and 4.5 g VS per litre. In case of grass the biogas production increased with increasing VS concentrations with methane yield of 199, 250, 256, 284, and 332 ml CH₄ per g VS at 3, 3.5, 4, 4.5, and 5.0 g VS per litre. The anaerobic biodegradability of coconut oil cake was evaluated in fed batch mode in a 5L anaerobic reactor at 4 g VS per litre per batch, and the maximum methane yield was found to be 320ml CH₄ per g VS.

Dubrovskis *et al.* (2012) studied the potential of biogas production from various raw materials, using available and cheaper raw materials. Biomass mixed with inoculum (fermented cow manure) was investigated for biogas production. Finally concluded that good quality silage, chopped oat bran and pigs manure can be used as feed stock for efficient biogas production. The methane yield of 0.303 l g⁻¹ DOM is a significant result in comparison with a theoretical optimum. The methane yield 0.342 l g⁻¹ DOM obtained from the dry fraction of pigs manure is a good result.

Eze and Ojike (2012) concluded that the anaerobic digestion though maize chaffs, stalks and cobs have the potential to generate biogas and the chaffs produced more biogas than the other two wastes. It was also observed that the chaffs generated more methane gas than the other two. Maize chaffs, stalks and cobs were shredded and mixed in water to waste ratios ranging from 3:1 to 4:1 (water to wastes) and anaerobically digested in three separate 0.1 m³ digesters for 30 days.

Meggye and Nagy (2012) conducted experiments using mixtures of liquid pig manure and plant additives to produce biogas and intensify biogas yield, and then gas engine tests were done for the energy utilization. Pig manure alone gives biogas with 52 per cent methane and when plant additives were added then it gives biogas with 62.5 to 77.1 per cent methane.

Narayani *et al.* (2012) explained that the cumulative bio gas production increased, when fruit wastes along with co substrates were used. Highest biogas production (405 ml) was obtained for 75 per cent fruit wastes + 25 per cent cow dung sample, whereas lowest for the sample 75 per cent fruit wastes + 25 per cent rice bran. Also the methane per cent was highest when the fruit wastes digested with cow dung (80 per cent) compared to the other samples. Anaerobic digestion with cow dung has

greater potential for biogas production because the presence of anaerobes that lead to enhancement of biogas production. Rice bran and fruit wastes alone does not have potential for biogas production.

Rajput *et al.* (2012) found that waste generated in the biogas power plant was harmful not as economic point of view but it was highly polluting and hazardous for human beings. However this waste can be further anaerobically digested and that will not only be eco-friendly but also it will generate biogas as high quality manure. For rapid digestion it has been found that slurry with PW in 60:40 leads high gas yield. Methane content in the generated biogas was also more than 47 per cent which is sufficient to run gas engines in power plant. Maximum gas generation was recorded on 60th day i.e. about (0.45 l g⁻¹ VS.).

Ilaboya *et al.* (2010) conducted a survey to ascertain the amount of biogas that can be generated from various feed stock. Cattle dung, poultry droppings and pig dung were used as substrates and the biogas yield was 0.3, 0.5 and 0.5 m³ kg⁻¹ respectively.

Fantozzi *et al.* (2009) designed a single-stage, batch, mixed, laboratory anaerobic digester to evaluate biogas production from different substrates. Good productivity for pig manure was found 0.13 Nm³ kg⁻¹VS and a high reduction of nitrogen content (poultry litter: 86 %). The influence of inoculum was also evaluated, when considering pig manure anaerobically digested and chicken manure the highest methane production was found (0.11 Nm³ kg⁻¹VS). When comparing pig manure to rumen fluid on olive husk, the first shows better performance (0.11 vs. 0.03 Nm³ kg⁻¹VS).

Sagagi *et al.* (2009) explained the results of the study on biogas production from fruits and vegetables waste materials and their effect on plants when used as fertilizer. It has been observed that the highest weekly individual production rate was recorded for the cow dung (control) slurry with average production of 1554 cm³, followed by pineapple waste which had 965 cm³ of biogas, then by orange waste which had 612 cm³ of biogas, lastly, pumpkin and spinach wastes had 373 cm³ and 269 cm³ respectively. The results obtained shows that difference in the production of biogas to a large extent depends on the nature of the substrate. All the substrates used appeared to be good materials for biogas production and their spent slurries can be used as a source of plant nutrients.

Singh *et al.* (2008) explained the volatile solids content of the poultry slurry was found to range from 35.6 per cent to 80.7 per cent, the average value being 61.10 per cent. With the daily feed of 42 kg poultry waste 3,000 litres of biogas was produced

per day. This could be an additional benefit for poultry industries in Kathmandu and also the whole Nepal in suitable environment. At the present poultry waste producing capacity of the farm of 93,075 kg per year the biogas generation could be estimated to 6,648 m³ per year. This could generate 36.96 MWh of thermal energy per year. The retention time was 82 days which was longer than cow dung digestion time which generally is around 40 to 50 days. The study also found that the odour level of poultry waste was perceived more in wet condition than when it was in dry form.

Stalin *et al.* (2007) developed a modified three stage methane fermentation system to digest animal manure effectively. The digester having an effective volume of 200 l was constructed with central tube filled with burnt bricks. The burnt brick in the central portion of the digester increase the microbial concentration by immobilizing the bacteria on the surface of the burnt brick. The carrier materials used in the digester were 5 per cent, 10 per cent, 15 per cent, and 20 per cent of the total volume of the digester and also for each percentage 3.5 kg of cow dung and 3.5 kg of water (1:1) is well mixed and added daily. The readings were taken between biogas generations versus time for each percentage continuously up to 90 days. It was observed that 10 to 15 percentage of carrier material from the total volume for microbial growth gave more gas generation.

Singh and Malik (2002) concluded that after conducting the experiment for the feasibility of generating biogas from press mud and from press mud + cattle waste mixed slurry and concluded that it is feasible to generate biogas from press mud alone at ambient temperature, and generate organic fertilizer for higher crop productivity at the same time.

Sathianathan, (1975) explained that higher the volatile solid content in a unit volume of fresh dung, the higher the gas production. For example, a kg of volatile solids in cow dung would yield about 0.25 m³ biogas.

2.2. THE VIABILITY OF BENEFICIAL MICROORGANISMS IN THE BIOGAS MANURES.

Shruthi *et al.* (2014) did work on the survivability of *Bacillus megatherium* in different carrier material for improved shelf life of biofertilizer. The survivability of *Bacillus megatherium* in different carrier material like press mud, coco peat, vermicompost and lignite was assessed by storing at room temperature for 240 days. The microbial population was estimated once in 30 days upto 240 days of storage (13.3×10^7 CFU g⁻¹ at 30 days and 2×10^7 CFU g⁻¹ at 240 days). Maximum survival of the organism was observed in press mud based biofertilizer because it was a rich source of

nutrients especially carbon which favours the microorganisms survivability, followed by vermicompost, then lignite and was lowest in cocopeat.

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. The studies concluded that there was significant improvement in the microbial population and nutrient concentration (N, P, K and micronutrients) of organic manures due to microbial enrichment. Among the enriched manures, enriched vermicompost had significantly highest microbial load, N, P, K and micronutrient content followed by enriched compost and biogas slurry. Microbial population in the slurry before enrichment was 80.5×10^5 bacteria, 124×10^3 fungi, 12×10^2 actinomycetes, 15.3×10^3 phosphate solubilising micro organisms and 20.2×10^3 free living nitrogen fixers. Where as after enrichment the microbial population was 102.2×10^5 , 162×10^3 , 19×10^2 , 25.8×10^3 and 33.1×10^3 respectively. Thus it can be concluded that there was a significant improvement in the microbial population after enrichment of the biogas slurry.

Karmegam and Rajashekar (2012) conducted research on the enrichment of biogas slurry vermicompost with *Azotobacter chroococcum* and *Bacillus megatherium*. The inoculum level of *Azotobacter chroococcum* and *Bacillus megatherium* at rate of 35 ml per 175 g of vermibed substrate is sufficient to maintain 1×10^7 viable cells up to 160 days after harvesting of vermicompost. The inoculum of biofertilizer organisms in vermibed on 30th day showed increased survival rate and hence, the optimized inoculation of 35 ml of inoculum per 175 g of substrate on 30th day of vermicomposting is helpful for maintenance of sufficient viable population for more than five months in the enriched vermicompost.

Rajasekar *et al.* (2012) studied the possibility of enrichment of vermicompost with microbial inoculants (i.e., biofertilizer organisms), *Azospirillum brasilense* and *Rhizobium leguminosarum*, optimization of inoculum level, and time of inoculation during vermicomposting. The survival rate of each microbial inoculant, total microbial population in vermicompost, and their correlation with the microbial inoculants during the storage period (180 days) were assessed. The change in population of *A. brasilense* and *R. leguminosarum* in vermicompost (at 30, 35, and 40 ml per 175 g substrates) with reference to storage period showed highly significant negative correlation ($P < 0.001$). The total microbial population in *A. brasilense* and *R. leguminosarum* inoculated vermicompost was high during initial phases of storage and then total microbial population declined towards the end (storage period 0 to 180 days).

Madan and Singh (2010) evaluated the nitrogen efficiency of different local fruit isolates of *Azotobacter* and evaluated the effect of vermicompost as carrier materials in the place of lignite. Among different isolates AZ 3 (14.0 mg N g⁻¹ sucrose or 14.0 mg N ml⁻¹ in culture medium) and AZB (13.49 mg N g⁻¹ sucrose or 13.49 mg N ml⁻¹ in culture medium) from rhizosphere of jackfruit and guava respectively fixed significantly higher nitrogen than others. The survival capacity of *Azotobacter* isolates in sterilized vermicompost and vermicompost plus FYM carrier recorded maximum value of 32.70x10⁸ CFU g⁻¹ and 28x10⁸ CFU g⁻¹ of dry weight from 90 to 120 days and gradually declined to 3.07x10⁸ and 0.50x10⁸ CFU g⁻¹ of dry weight on 240 days of storage, respectively.

Rajashekar and Karmegam (2010) studied the use of biogas slurry for the preparation of vermibed. Vermicasts and lignite were mixed in various proportions and used as carrier substrate for biofertilizers (*Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum*). The viable count in the stored carrier material was individually carried out once in 15 days for a total period of 10 months. During the initial 2–4 weeks, slight increase in viable counts of *Azotobacter chroococcum* were seen, later on gradual decline with intermittent increase and at the end of 10th month progressive decline in viable cells were recorded. The survival rate of *Bacillus megaterium* in different ratio of carrier materials showed decline upon the incubation. Similar to *Azotobacter chroococcum*, *Bacillus megaterium* also exhibited increased rate of survival when the proportion of vermicasts in carrier material increased.

Muthuselvam and Tholkappian (2008) used the vermicompost as a potential carrier material for bacterial inoculants. Total four carrier materials viz., lignite, vermiculite, sterilized vermicompost and raw vermicompost were assessed for survival and use as carrier material for biofertilizers such as *Azospirillum*, *Azotobacter*, *Rhizobium* and phosphobacteria. The results showed raw vermicompost can be a potential carrier material than other materials used.

Priya *et al.* (2008) evaluated the effect of inoculation of nitrogen fixing *Azotobacter chroococcum* strain; *Azospirillum brasilense* strain and phosphate solubilizing *Pseudomonas maltophilia*, on nitrogen and phosphorus content of vermicomposts prepared from cow dung and cow dung spiked textile mill sludge. The cow dung vermicompost was more supportive to the growth and multiplication of all the three bacteria than cow dung spiked textile mill sludge vermicompost. In *Azotobacter chroococcum* treated vermicomposts maximum nitrogen content was recorded between

45 and 60 days [cow dung vermicompost ($25.9 \pm 0.45 \text{ g kg}^{-1}$) and cow dung spiked textile mill sludge vermicompost ($20.6 \pm 0.62 \text{ g kg}^{-1}$)] followed by *Azospirillum brasilense* inoculation [cow dung vermicompost ($19.4 \pm 0.60 \text{ g kg}^{-1}$) and cow dung spiked textile mill sludge vermicompost ($18.6 \pm 0.17 \text{ g kg}^{-1}$)]. Phosphorus content in *Pseudomonas maltophilia* inoculated cow dung vermicompost was $20.8 \pm 0.20 \text{ g kg}^{-1}$ and cow dung spiked textile mill sludge vermicompost was $13.4 \pm 0.45 \text{ g kg}^{-1}$ after 75th day of inoculation.

Kavitha and Subramanian (2007) studied the transformation of the normal compost into bioactive compost through the addition of various substrates (compost enriched with composted poultry litter, spent wash and rock phosphate) and microbial inoculants (*Azotobacter*, *Pseudomonas*, and *Phosphobacteria*). The highest nitrogen content (1.75 per cent), phosphorus content (1.16 per cent) and beneficial microorganisms viz., *Azotobacter*, *Pseudomonas*, *phosphobacteria* were observed in the treatment T₅ (compost enriched with composted poultry litter, spent wash, microbial inoculants and rock phosphate). Beneficial microorganisms, composted poultry litter, spent wash, and rock phosphate contributed maximum level of nutrients and growth promoters to the compost with small expenditure.

Chapter III

MATERIAL AND METHODS

The material and the methods adopted during the course of investigation are presented in this chapter. Analysis was conducted in the Department of Agricultural Microbiology and Bio-energy, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad.

The general laboratory techniques followed in the present study were those described by Cappuccino and Sherman (1992) and Aneja (2001) for preparation of media, sterilization, isolation and enumeration of microorganisms, with slight modifications wherever necessary.

3.1 COLLECTION OF DIFFERENT SUBSTRATES FOR SETTING UP OF BIOGAS UNITS ON LAB SCALE.

Different substrates were collected from different places and biogas digesters were set in lab scale. Press mud was collected from Nandyal sugar factory located at Nandyal, Kurnool district and cow dung was collected from the cattle farm of Veterinary University, Rajendranagar, Hyderabad. Poultry litter was collected from poultry farm of Veterinary University, Rajendranagar, Hyderabad. Kitchen wastes were collected from the mess of hostel-C. Maize stalks were collected from the college farm of ANGRAU, Hyderabad. Fruit wastes were collected from the fruit market at Rajendranagar, Hyderabad. Cow dung was collected from the cattle farm of Veterinary University, Rajendranagar, Hyderabad.

Table 3.1 Details of the treatments.

Treatment	Substrate
T ₁	Press mud + Cow dung
T ₂	Poultry litter + Cow dung
T ₃	Kitchen wastes + Cow dung
T ₄	Maize stalks + Cow dung
T ₅	Fruit waste + Cow dung
T ₆	Cow dung alone

For each treatment 750 g substrate and 1500 ml water (1:2) was used for the biogas production. In T₁ 250 g cow dung + 500 g press mud + 1500ml water (1:2:6) was used, in T₂ 250 g cow dung + 500 g poultry litter + 1500 ml water (1:2:6) was used, in T₃ 250 g cow dung + 500 g kitchen waste + 1500 ml water (1:2:6) was used, in T₄ 250

g cow dung + 500 g maize stalks + 1500 ml water (1:2:6) was used, in T₅ 250 g cow dung + 500 g fruit waste + 1500 ml water (1:2:6) was used and in T₆ 750 g cow dung + 1500 ml water (3:6) was used.

3.2 GLASSWARE:

Glass bottles of 3 litres capacity were used to set biogas anaerobic digester in the laboratory scale. Petri plates, test tubes, microscopic slides, conical flasks of different capacities *i.e.*, 1000, 500 and 250 ml, Pipettes of 1, 2.2, 5 and 10 ml, beakers and measuring cylinders of 50, 100, 500, 1000 and 2000 ml were used. For estimation of organic carbon, BOD and COD, 50 ml burette was used.

3.2.1 Cleaning of Glassware:

Glassware were first washed with a detergent, then cleaned with tap water and finally placed in the chromic acid solution prepared with following composition:

Potassium dichromate - 60 g, Conc.H₂SO₄ - 60 ml, Distilled water - 1000 ml

The glassware were kept in the cleaning solution for 24 h and then thoroughly washed with running tap water before its final cleaning with distilled water and oven dried.

3.2.2 Sterilization of glassware and media

Glassware like pipettes were sterilized in the hot air oven at 160⁰C for 1 1/ 2 h before use. Petri plates, test tubes with saline and media were used in the experiment to estimate the microbial counts. The media like Yeast Extract Mannitol Agar medium, Azotobacter glucose medium, Potato Infusion Agar medium etc. and distilled water were sterilized in an autoclave at 15 lb psi (121⁰C) for 20 min.

3.3 EQUIPMENT AND APPARATUS USED

Hot air oven and autoclaves were used for sterilization of heat stable materials like pipettes and media respectively. Laminar air flow chamber and plate mixer were used for spread plate technique. BOD incubators were used for incubating the samples in BOD bottles to estimate the BOD in the slurry samples. COD apparatus was used for estimating COD in the slurry samples. The pH was measured by using digital pH meter. Elico CM 180 conductivity meter was used to estimate the E.C in the slurry samples. Cyclomixer was used for homogenization during serial dilution. Mechanical shaker for shaking the broth tubes during the experimental procedures. Kjeldhal apparatus was used to estimate N in the slurry samples. Spectrophotometer was used to estimate the P content in the samples. Flame photometer was used to estimate K content in the slurry samples. Muffle furnace was used to estimate the total volatile solids in the slurry samples.

3.4 CHEMICALS USED

The chemicals used in the present investigation were of analytical grade. The pH of the media was adjusted to the required level using 1N NaOH and 1N HCl. Formaldehyde 10 per cent solution was used to fumigate the laminar air flow chamber and BOD incubator for disinfection.

3.4.1 PREPARATION OF MEDIA

3.4.1.1 Yeast Extract Mannitol with Congo Red medium (YEM agar)

Rhizobium medium was prepared by taking 31.83 g Yeast Extract Mannitol with Congo Red medium (Himedia) and dissolving in 1000 ml of water and sterilized in an autoclave at 15 psi (121°C) for 15 min.

3.4.1.2 King's B medium

King's B medium was prepared by taking 42.23 g King's Medium B Base in 1000 ml distilled water and sterilized in an autoclave at 15 psi (121°C) for 15 min.

3.4.1.3 Azotobacter glucose medium

Azotobacter glucose medium (Himedia) was prepared by taking 31.48 g of the Azotobacter glucose medium and mixed in 1000 ml of distilled water and sterilized in an autoclave at 15 psi (121°C) for 15 min.

3.4.1.4 Potato infusion agar medium

Potato infusion agar medium was prepared by taking 49.0 g of the potato infusion agar medium and mixed in 1000 ml of distilled water and sterilized in an autoclave at 15 psi (121°C) for 15 min.

3.5 LABORATORY ANALYSIS

Analysis of biogas slurry samples

3.5.1 Total solids percentage (%) in the slurry samples

Total solids per cent in the slurry samples was determined by drying a 100g of sample for 105° C for 24 h. (APHA, 1992).

Total solids (TS) per cent = $\{(\text{Weight of dried sample} + \text{weight of crucible}) - (\text{weight of the crucible})\} / \text{weight of the sample as received} \times 100$.

3.5.2 Total volatile solids percentage (%) in the slurry samples

Total volatile solids per cent in the slurry samples was determined by incinerating the dry sample at 550° C for 2 h. (APHA, 1992).

Total volatile solids (TVS) per cent = $\{(\text{Weight of dried sample} + \text{weight of crucible}) - (\text{weight of the crucible} + \text{weight of sample after ignition})\} / \{(\text{weight of wet sample} + \text{weight of crucible}) - (\text{weight of the crucible})\} \times 100$.

3.5.3 Volatile fatty acids in the slurry samples

Volatile fatty acids in the slurry samples was estimated by titration method (Standard methods, 1985).

3.5.4 pH in the slurry samples

pH of the slurry samples was determined in 1:2.5 substrate water suspension by using digital pH meter (Systronics μ pH system 361) (Jackson, 1973).

3.5.5 Electrical conductivity (E.C) in the slurry samples

The electrical conductivity of slurry samples was determined in saturation extract by using Elico CM 180 conductivity meter (Jackson, 1973).

3.5.6 N,P,K in the slurry samples

3.5.6.1 Nitrogen

Total nitrogen in slurry samples was estimated by modified Kjeldahl method using sulphuric and salicylic acid mixture. One gram of slurry sample was taken into 100 ml conical flask, 30 ml of sulphuric acid - salicylic acid mixture and 0.5 g of sodium thiosulphate was added, mixed well and kept aside for half an hour and digested on flame. After 30 min of digestion, one gram of copper sulphate and 10 g of potassium sulphate was added, digestion was continued till colourless solution obtained.

The digested material was washed with distilled water and only supernatant liquid was transferred to a beaker. From the beaker the solution was transferred to kjeldhal flask. 50 ml of 4 per cent boric acid taken into 250 ml conical flask to which two drops of mixed indicator was added and kept at the flask at the receiving end of distillation set in such a way that the receiving end immersed into the solution. Few Zn pieces, little quantity of paraffin and 120 ml of 40 per cent NaOH was added to the Kjeldhal flask and immediately mouth of the flask was closed. The distillation continued till no more ammonia was evolved at the receiving end of the distillation set. At the end of distillation, the tip of receiving end was washed with distilled water, contents of the flask were cooled and titrated against 0.1 N H_2SO_4 till blue colour changed to pinkish red colour. Burette reading was noted and N per cent was calculated as:

Weight of sample taken for analysis = w grams

Volume of 0.1 N H_2SO_4 utilised = Y ml

1 ml of 0.1 N H_2SO_4 = 0.0014 g of N

$$\begin{aligned}
\text{So } Y \text{ ml } 0.1 \text{ N H}_2\text{SO}_4 &= Y \times 0.0014 \text{ g of N} \\
W \text{ g sample contains} &= Y \times 0.0014 \text{ g of N} \\
100 \text{ g sample contains} &= (Y \times 0.0014 \times 100) / w \\
\text{N per cent} &= (Y \times 0.0014 \times 100) / w
\end{aligned}$$

3.5.6.2 Phosphorus

Total phosphorus content in slurry samples was determined by perchloric acid digestion method using Barton's reagent as described by Jackson (1967). One gram of slurry sample was taken into 100 ml conical flask and 12-15 ml of triacid mixture was added (Nitric acid: Sulphuric acid: Perchloric acid at 9:2:1). The mouth of the flask was covered with a funnel. The contents were digested over a sand bath till clear solution was obtained. The filtrate was collected and 5 ml was taken into 25 ml volumetric flask and 5 ml of Barton's reagent was added and volume made upto 25 ml with distilled water. Yellow colour developed in 30 minutes and intensity of colour was measured in a photoelectric colorimeter using blue filter (470 nm). The colour will be stable for 24 h. Standard curve was prepared and the concentration of P in the solution was deduced from that value and the percentage of P in the sample was calculated.

Amount of P present in the sample (% of P) = $(A/10^6) \times (25/5) \times (100/1) \times 100 \times 100 / (100-M)$.

Concentration of P read in the standard curve = $A \text{ mg ml}^{-1}$.

3.5.6.3 Potassium

Triacid extract was directly aspirated to the flame photometer to estimate the total potassium content (Systronics flame photometer 128) by Jackson (1967). 5 ml of triacid extract was taken into 25 ml volumetric flask and volume made upto the mark with distilled water. The concentration of K in the solution was measured using flame photometer. Standard curve was prepared and the concentration of K in the solution was deduced from that value and the percentage of K in the sample was calculated.

Amount of K present in the sample (% of K) = $(A \times 25 \times 100 \times 100) / (10^6 \times 5 \times 1) \times 100 / (100-M)$

Concentration of K read in the standard curve = $A \text{ mg ml}^{-1}$.

3.5.7 Organic Carbon percentage in the slurry samples

Organic carbon content of the slurry samples was estimated by Walkley and Black's wet oxidation method as outlined by Walkley and Blacks (1934). One gram of slurry sample was taken into 500 ml conical flask and to it 10 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ and 20 ml of conc H_2SO_4 was added. Then kept on an asbestos sheet for 30 min and 200 ml of distilled water and 10 ml of ortho phosphoric acid was added. Diphenylamine indicator

was added and titrated with 0.5 N ferrous ammonium sulphate solution until green colour appearing. A blank was run along with the sample.

Organic carbon per cent in slurry sample = $10 (B-S) / B \times 0.003 \times 100 / \text{wt of the sample taken (g)}$.

Titre value of the blank in ml = B

Titre value of the sample in ml = S

3.5.8 BOD in the slurry samples

Biological Oxygen Demand was determined by measuring oxygen concentration in slurry sample, idometrically before and after incubation in the dark at 20⁰ C for 5 days by the colorimetric method (APHA, 1992). BOD bottles were taken and filled with the sample and stopper was placed. Then the stopper was removed and 1 ml of each manganous sulphate and alkaline potassium sulphate were added. Precipitate formed. 2 ml of sulphuric acid was added to dissolve the precipitate. Then the whole content was transferred into a conical flask. Few drops of starch indicator was added and titrated against sodium thiosulphate solution till the initial blue colour turned to colourless. The titre value obtained gives dissolved Oxygen (D.O₁)

Dissolved Oxygen (D.O₁) = $(V_1 N \times 8 \times 1000) / (V_2 - V_3)$.

Volume of titrant (ml) = V₁

Volume of sampling bottle after placing the stopper (ml) = V₂

Volume of manganous sulphate and potassium sulphate solution added (ml) = V₃

Other set of BOD bottles were prepared same as above and incubated in BOD incubator for 5 days. After 5 days the content from BOD bottles was transferred into conical flask and titrated similarly. The dissolved oxygen content D.O₅ was determined.

BOD = $(D.O_1 - D.O_5) \times \text{dilution factor}$.

3.5.9 COD in the slurry samples

Chemical Oxygen Demand was determined by taking centrifuged slurry samples and refluxed with a known amount of potassium dichromate in sulphuric acid medium and excess of dichromate was titrated against ferrous ammonium sulphate. The amount of dichromate consumed was proportional to the oxygen required to oxidize the organic matter. Chemical oxygen demand was determined by the closed reflux colorimetric method (APHA, 1992).

COD = $(B - T) \times N \times 1000 \times 8 / \text{Volume of sample (ml)}$

3.5.10 Measurement of Gas production from different treatments

The biogas production readings were taken on an alternate day by water displacement method in the measuring jar.

3.5.11 Estimation of methane percentage using gas chromatography

Methane percentage in the biogas was estimated using gas chromatography (Bruker-450 GC) with a flame ionization detector (FID). Temperatures were maintained at 300°C in the detector, 75°C in the injector and 50°C in the oven. The column used was porapak Q. The gas flow in the column was maintained as 60 ml min⁻¹.

3.6 To evaluate the biogas slurry for the viability of beneficial microorganisms.

3.6.1 Collection of Biogas slurry

The biogas slurry was collected from different treatments and dried (50 % moisture) under the sun. The dried slurry was utilized to evaluate the viability of added beneficial microorganisms.

3.6.2 Microbial Analysis of Biogas slurry

The dried biogas slurry collected from different treatments was analysed for the presence of microorganisms i.e., total bacteria, *Rhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas* by serial dilution and plating on selective media as mentioned above. Replicates of the inoculated agar plates were incubated for 2 days at 37°C for *Rhizobium* and *Pseudomonas*, 7 days for *Azotobacter* and *Azospirillum* after which the counts were taken.

3.7 Viability of added beneficial microbes in Biogas slurry

3.7.1 Collection of Beneficial micro organisms

Beneficial microorganisms *Rhizobium*, *Pseudomonas*, *Azotobacter*, *Azospirillum* cultures obtained from the Agricultural Microbiology Department, College of Agriculture, Rajendranagar, were used in the experiment.

3.7.2 Enrichment of Biogas slurry

The dried biogas slurry samples collected from different treatments after drying were enriched with 4 types of biofertilizers viz: *Rhizobium*, *Azotobacter*, *Azospirillum* and *Pseudomonas* each separately and also altogether.

3.7.3 Microbial Analysis of Biogas slurry Enriched with Beneficial Microorganisms

Viable population of *Rhizobium*, *Pseudomonas*, *Azotobacter*, *Azospirillum* were analyzed by the standard serial dilution plate count method (Vlassak *et al.*, 1992) at weekly interval in the first month and monthly interval from the second month by using different Media viz: Yeast Extract Mannitol Agar with Congo Red for *Rhizobium*, Kings-B for *Pseudomonas* (King *et al.* 1954), Azotobacter medium for *Azotobacter* spp., Potato infusion agar medium for *Azospirillum* spp., and plates were incubated at

28±2 °C in an incubator in triplicates. The microbial colonies appearing after the stipulated time period of incubation were counted as Colony forming units (CFU) g⁻¹ fresh weight of the sample. The microbial populations were expressed as number of colony forming units per gram.

3.8 STATISTICAL ANALYSIS

The analysis and interpretation of data were done using the Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The levels of significance used in 'F' test and 't' test was P = 0.05 and critical values were calculated wherever the 'F' test was significant.

Chapter IV

RESULTS AND DISCUSSION

The results of the two experiments under the title “Biogas production from agricultural wastes and feasibility study to enrich biogas manures” are shown in this chapter which was conducted in Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar.

Different agricultural wastes along with cow dung were used for the anaerobic digestion in the lab scale for optimization of biogas production and biogas manures were enriched with beneficial microorganisms and the viability was observed.

4.1 OPTIMIZATION OF METHANE GAS PRODUCTION FROM COW DUNG ALONG WITH OTHER AGRICULTURAL WASTES.

4.1.1 Biogas production

All the biogas production units with six treatments and three replications were set on the same day with 750 grams substrate and 1500 ml water (1:2 ratio).

By the end of first week, the gas evolved was significantly more in T₁ (Cow dung + Press mud) 1110.00 ml, compared to T₅ (Cow dung + Fruit waste) 520.00 ml, T₆ (Cow dung alone) 370 ml, T₂ (Cow dung + Poultry litter) 353.33 ml, T₃ (Cow dung + Kitchen waste) 313.33 ml and less in T₄ (Cow dung + Maize stalks) 213.33 ml (Table 4.2).

By the end of second week, the gas evolved was significantly more in T₁ (Cow dung + Press mud) 1236.67 ml, compared to T₄ (Cow dung + Maize stalks) 920.00 ml, T₅ (Cow dung + Fruit waste) 680.00 ml, T₃ (Cow dung + Kitchen waste) 623.33 ml, T₆ (Cow dung alone) 523.33 ml and less in T₂ (Cow dung + Poultry litter) 383.33 ml (Table 4.2).

By the end of third week, the gas evolved was significantly more in T₁ (Cow dung + Press mud) 1313.33 ml, compared to T₆ (Cow dung alone) 1240.00 ml, T₃ (Cow dung + Kitchen waste) 1123.33 ml, T₅ (Cow dung + Fruit waste) 840.00 ml, T₄ (Cow dung + Maize stalks) 683.33 ml and less in T₂ (Cow dung + Poultry litter) 460.00 ml (Table 4.2).

By the end of fourth week, the gas evolved was significantly more in T₆ (Cow dung alone) 1380.00 ml, as compared to T₁ (Cow dung + Press mud) 1353.33 ml, T₂ (Cow dung + Poultry litter) 1276.67 ml, T₃ (Cow dung + Kitchen waste) 723.33 ml, T₅ (Cow dung + Fruit waste) 563.33 ml and less in T₄ (Cow dung + Maize stalks) 503.33 ml (Table 4.2).

By the end of fifth week, the gas evolved was significantly more T₆ (Cow dung alone) 1523.33 ml, compared to T₁ (Cow dung + Press mud) 1483.33 ml, T₂ (Cow dung + Poultry litter) 1380.00 ml, T₃ (Cow dung + Kitchen waste) 540.00 ml, T₅ (Cow dung + Fruit waste) 240.00 ml and less in T₄ (Cow dung + Maize stalks) 203.33 ml (Table 4.2).

By the end of sixth week, the gas evolved was significantly more T₁ (Cow dung + Press mud) 1380.00 ml, compared to T₆ (Cow dung alone) 1280.00 ml, T₂ (Cow dung + Poultry litter) 886.66 ml, T₃ (Cow dung + Kitchen waste) 416.66 ml, T₄ (Cow dung + Maize stalks) 183.33 ml and less in T₅ (Cow dung + Fruit waste) 150.00 ml (Table 4.13).

By the end of seventh week, the gas evolved was significantly more T₁ (Cow dung + Press mud) 1076.67 ml, compared to T₆ (Cow dung alone) 966.66 ml, T₂ (Cow dung + Poultry litter) 616.66 ml, T₃ (Cow dung + Kitchen waste) 123.33 ml, T₄ (Cow dung + Maize stalks) 150.00 ml and less in T₅ (Cow dung + Fruit waste) 150.00 ml (Table 4.2).

By the end of eighth week, the gas evolved was significantly more T₁ (Cow dung + Press mud) 586.66 ml, compared to T₆ (Cow dung alone) 563.33 ml, T₂ (Cow dung + Poultry litter) 563.33 ml, T₄ (Cow dung + Maize stalks) 123.33 ml, T₅ (Cow dung + Fruit waste) 106.66 ml and less in T₃ (Cow dung + Kitchen waste) 76.66 ml (Table 4.2).

By the end of ninth week, the gas evolved was significantly more T₁ (Cow dung + Press mud) 266.66 ml, compared to T₆ (Cow dung alone) 176.66 ml, T₂ (Cow dung + Poultry litter) 120.22 ml, T₄ (Cow dung + Maize stalks) 106.66 ml, T₅ (Cow dung + Fruit waste) 90.00 ml and less in T₃ (Cow dung + Kitchen waste) 26.66 ml (Table 4.2).

By the end of tenth week, the gas evolved was significantly more T₁ (Cow dung + Press mud) 96.66 ml, compared to T₆ (Cow dung alone) 80.00 ml, T₅ (Cow dung + Fruit waste) 60.00 ml, T₂ (Cow dung + Poultry litter) 40.00 ml, T₄ (Cow dung + Maize stalks) 20.00 ml and less in T₃ (Cow dung + Kitchen waste) 13.33 ml (Table 4.2).

The highest gas production was observed in T₁ (Cow dung + Press mud) 9903.31 ml, as compared to T₆ (Cow dung alone) 8103.31 ml, T₂ (Cow dung + Poultry litter) 6079.98 ml, T₃ (Cow dung + Kitchen waste) 4066.63 ml, T₅ (Cow dung + Fruit waste) 3373.32 ml and less in T₄ (Cow dung + Maize stalks) 3099.97 ml.

Singh *et al.* (2008) explained the volatile solids content of the poultry slurry was found to range from 35.6 per cent to 80.7 per cent, the average value being 61.10 per cent. With the daily feed of 42 kg poultry waste 3,000 litres of biogas was produced per day.

Sagagi *et al.* (2009) explained the results of the study on biogas production from fruits and vegetables waste materials and their effect on plants when used as fertilizer. It has been observed that the highest weekly individual production rate was recorded for the cow dung (control) slurry with average production of 1554 cm³, compared to pineapple waste which had 965 cm³ of biogas, then by orange waste which had 612 cm³ of biogas, lastly, pumpkin and spinach wastes had 373 cm³ and 269 cm³ respectively. The results obtained shows that difference in the production of biogas to a large extent depends on the nature of the substrate.

Ofoefule *et al.* (2010) stated cow dung produced highest amount of biogas while goat dung produced less volume of biogas. Anaerobic digestion of some animal dung like cow, swine, rabbit, poultry and goat was carried out. The average volume of biogas produced was 44, 40, 37, 33 and 31 dm³ per total mass of slurry respectively.

4.1.2 Total solids (TS) per cent

Total solids (TS) per cent in the substrate was significantly more in T₆ (Cow dung alone) 18.93 per cent compared to T₁ (Cow dung + Press mud) 18.76 per cent, T₅ (Cow dung + Fruit waste) 16.5 per cent, T₂ (Cow dung + Poultry litter) 14.43 per cent, T₄ (Cow dung + Maize stalks) 11.26 per cent and less in T₃ (Cow dung + Kitchen waste) 11.13 per cent (Table 4.1).

In the above result, total solids per cent in the kitchen waste was similar to the results of Velmurugan *et al.* (2011) who demonstrated that vegetable wastes (Banana stem, Cabbage and Ladies finger) were anaerobically digested in a fed-batch laboratory scale reactor at mesophilic conditions (35⁰C). The total solids per cent in the substrates were estimated as 6.55 per cent, 8.50per cent and 12.28 per cent in banana stem, cabbage, ladies finger respectively.

The results obtained were similar to the observations of Ofoefule *et al.* (2010) who carried anaerobic digestion of animal dung like cow, swine, rabbit, poultry and goat. The total solids per cent in the animal dung was 36.38 per cent, 27.01 per cent, 15.65 per cent, 17.02 per cent and 19.89 per cent respectively.

In the results T₆ (Cow dung alone) had more total solids per cent compared to the other treatments and this results were similar to the work of Rouf *et al.* (2010) who carried the optimization of biogas generation from press mud, cow dung and kitchen wastes using batch reactor. Press mud used for biogas generation contain 22.2 per cent total solids, cow dung contains 24.9 per cent TS and kitchen wastes contain 11.4 per cent TS.

In the inoculum mixture (on zero day) the total solids (TS) per cent was significantly more in T₆ (Cow dung alone) 18.06 per cent compared to T₁ (Cow dung + Press mud) 17.73 per cent which was on par and others T₅ (Cow dung + Fruit waste) 15.3 per cent, T₂ (Cow dung + Poultry litter) 13.1 per cent, T₄ (Cow dung + Maize stalks) 10.56 per cent and least in T₃ (Cow dung + Kitchen waste) 10.46 per cent (Table 4.3).

On 7th day the total solids (TS) per cent was significantly more in T₆ (Cow dung alone) 17.03 per cent compared to T₁ (Cow dung + Press mud) 14.36 per cent, T₅ (Cow dung + Fruit waste) 13.59 per cent, T₂ (Cow dung + Poultry litter) 12.46 per cent, T₃ (Cow dung + Kitchen waste) 9.73 per cent and less in T₄ (Cow dung + Maize stalks) 8.25 per cent (Table 4.3).

On 14th day the total solids (TS) per cent was significantly more in T₁ (Cow dung + Press mud) 13.46 per cent compared to, T₆ (Cow dung alone) 12.85 per cent, T₅ (Cow dung + Fruit waste) 12.80 per cent, T₂ (Cow dung + Poultry litter) 11.33 per cent, T₃ (Cow dung + Kitchen waste) 8.81 per cent and less in T₄ (Cow dung + Maize stalks) 7.71 per cent (Table 4.3).

On 21st day the total solids (TS) per cent was significantly more in T₁ (Cow dung + Press mud) 12.46 per cent compared to T₅ (Cow dung + Fruit waste) 12.44 per cent, T₆ (Cow dung alone) 10.86 per cent, T₂ (Cow dung + Poultry litter) 10.33 per cent, T₃ (Cow dung + Kitchen waste) 8.42 per cent and less in T₄ (Cow dung + Maize stalks) 7.51 per cent (Table 4.3).

On 28th day the total solids (TS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 11.82 per cent compared to T₁ (Cow dung + Press mud) 11.44 per cent T₂ (Cow dung + Poultry litter) 9.33 per cent, T₆ (Cow dung alone) 9.25 per cent, T₃ (Cow dung + Kitchen waste) 7.95 per cent and less in T₄ (Cow dung + Maize stalks) 7.23 per cent (Table 4.3).

On 42nd day the total solids (TS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 11.20 per cent compared to T₁ (Cow dung + Press mud) 9.81 per cent, T₆ (Cow dung alone) 8.56 per cent, T₂ (Cow dung + Poultry litter) 7.85 per cent, T₄ (Cow dung + Maize stalks) 7.01 per cent and less in T₃ (Cow dung + Kitchen waste) 6.82 per cent (Table 4.3).

By the end of the process (on 56th day) the degradation of total solids (TS) per cent was significantly more in T₆ (Cow dung alone) 57.16 per cent compared to T₁ (Cow dung + Press mud) 52.94 per cent, T₃ (Cow dung + Kitchen waste) 52.47 per cent,

T₃ (Cow dung + Kitchen waste) 44.93 per cent, T₄ (Cow dung + Maize stalks) 39.53 per cent and less in T₅ (Cow dung + Fruit waste) 32.55 per cent (Table 4.3).

4.1.3 Total volatile solids (TVS) per cent

Total volatile solids (TVS) per cent in the substrates was significantly more in T₅ (Cow dung + Fruit waste) 89.34 per cent compared to T₄ (Cow dung + Maize stalks) 83.21 per cent, T₂ (Cow dung + Poultry litter) 81.58 per cent, T₃ (Cow dung + Kitchen waste) 81.52 per cent, T₆ (Cow dung alone) 80.06 per cent and in T₁ (Cow dung + Press mud) 72.40 per cent (Table 4.1).

Velmurugan and Alwar Ramanujam (2011) demonstrated that the total volatile solids per cent in the substrates, vegetable wastes (banana stem, cabbage and ladies finger) were 83.21 per cent, 90.45 per cent and 90.65 per cent in banana stem, cabbage and ladies finger respectively.

Ofoefule *et al.* (2010) reported similar TVS per cent for animal dung like cow and poultry. The total volatile solids (TVS) per cent was 82.35 per cent and 83.80 per cent. The TVS per cent was significantly more in T₂ (Cow dung + Poultry litter) compared to T₆ (Cow dung alone).

Rouf *et al.* (2010) showed that press mud used for biogas generation contained 77.1 per cent total volatile solids, cow dung contained 77.2 per cent TVS and kitchen wastes had 92.0 per cent TVS. Our results T₃ (Cow dung + Kitchen waste) with more TVS per cent compared to T₆ (Cow dung alone) 80.06 per cent and T₁ (Cow dung + Press mud) is in conformation with above results.

The total volatile solids in the press mud (72.40 %) obtained in the results was similar to the observation of Agrawal *et al.* (2011) who explained that the press mud contains 77 per cent volatile solids which favours biogas production.

In the inoculum mixture (on zero day) the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 89.85 per cent compared to T₄ (Cow dung + Maize stalks) 84.20 per cent, T₂ (Cow dung + Poultry litter) 82.58 per cent, T₃ (Cow dung + Kitchen waste) 81.62 per cent, T₆ (Cow dung alone) 81.31 per cent and the least in T₁ (Cow dung + Press mud) 73.82 per cent (Table 4.5).

On 7th day the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 87.34 per cent compared to T₄ (Cow dung + Maize stalks) 82.02 per cent, T₂ (Cow dung + Poultry litter) 80.41 per cent, T₃ (Cow dung + Kitchen waste) 80.09 per cent, T₆ (Cow dung alone) 78.96 per cent and less in T₁ (Cow dung + Press mud) 70.36 per cent (Table 4.5).

On 14th day the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 86.16 per cent compared to T₃ (Cow dung + Kitchen waste) 78.90 per cent, T₄ (Cow dung + Maize stalks) 77.61 per cent, T₂ (Cow dung + Poultry litter) 75.82 per cent, T₆ (Cow dung alone) 75.69 per cent and less in T₁ (Cow dung + Press mud) 64.37 per cent (Table 4.5).

On 21st day the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 84.33 per cent compared to T₃ (Cow dung + Kitchen waste) 77.61 per cent, T₂ (Cow dung + Poultry litter) 74.84 per cent, T₄ (Cow dung + Maize stalks) 70.52 per cent, T₆ (Cow dung alone) 69.41 per cent and less in T₁ (Cow dung + Press mud) 63.86 per cent (Table 4.5).

On 28th day the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 83.58 per cent compared to T₃ (Cow dung + Kitchen waste) 76.24 per cent, T₂ (Cow dung + Poultry litter) 72.19 per cent, T₄ (Cow dung + Maize stalks) 68.20 per cent, T₆ (Cow dung alone) 64.81 per cent and less in T₁ (Cow dung + Press mud) 63.24 per cent (Table 4.5).

On 42nd day the total volatile solids (TVS) per cent was significantly more in T₅ (Cow dung + Fruit waste) 80.46 per cent compared to T₃ (Cow dung + Kitchen waste) 66.33 per cent, T₄ (Cow dung + Maize stalks) 66.24 per cent, T₂ (Cow dung + Poultry litter) 65.65 per cent, T₆ (Cow dung alone) 58.26 per cent and less in T₁ (Cow dung + Press mud) 57.42 per cent (Table 4.5).

By the end of the process (on 56th day) the degradation of total volatile solids (TVS) per cent was comparatively more in T₆ (Cow dung alone) 37.41 per cent compared to T₂ (Cow dung + Poultry litter) 35.21 per cent, T₃ (Cow dung + Kitchen waste) 26.01 per cent, T₁ (Cow dung + Press mud) 22.23 per cent, T₄ (Cow dung + Maize stalks) 19.34 per cent and less in T₅ (Cow dung + Fruit waste) 14.32 per cent (Table 4.5).

4.1.4 Total volatile fatty acids

In the substrate VFA concentration was significantly more in T₃ (Cow dung + Kitchen waste) 1.19 g l⁻¹ compared to T₁ (Cow dung + Press mud) 0.96 g l⁻¹, T₄ (Cow dung + Maize stalks) 0.80 g l⁻¹, T₂ (Cow dung + Poultry litter) 0.72 g l⁻¹, T₆ (Cow dung alone) 0.49 g l⁻¹ and less in T₅ (Cow dung + Fruit waste) 0.38 g l⁻¹ (Table 4.1).

Vedrenne *et al.* (2007) studied the effect of incubation conditions on the laboratory measurement of the methane producing capacity of cow dung. VFA in the cow dung was estimated as 0.3-7.4 g l⁻¹.

VFA concentration in the inoculum mixture (on zero day) was significantly more in T₃ (Cow dung + Kitchen waste) 1.25 g l⁻¹, compared to T₁ (Cow dung + Press mud) 1.03 g l⁻¹, T₄ (Cow dung + Maize stalks) 0.85 g l⁻¹, T₂ (Cow dung + Poultry litter) 0.78 g l⁻¹, T₆ (Cow dung alone) 0.61 g l⁻¹ and less in T₅ (Cow dung + Fruit waste) 0.48 g l⁻¹ (Table 4.5).

On 7th day VFA concentration was significantly more in T₂ (Cow dung + Poultry litter) 0.75 g l⁻¹, compared to T₄ (Cow dung + Maize stalks) 0.62 g l⁻¹, T₅ (Cow dung + Fruit waste) 0.51 g l⁻¹, T₁ (Cow dung + Press mud) 0.48 g l⁻¹, T₆ (Cow dung alone) 0.46 g l⁻¹ and less in T₃ (Cow dung + Kitchen waste) 0.43 g l⁻¹ (Table 4.5).

On 14th day VFA concentration was significantly more in T₄ (Cow dung + Maize stalks) 1.67 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 1.41 g l⁻¹, T₅ (Cow dung + Fruit waste) 1.13 g l⁻¹, T₂ (Cow dung + Poultry litter) 0.83 g l⁻¹, T₁ (Cow dung + Press mud) 0.53 g l⁻¹ and less in T₆ (Cow dung alone) 0.52 g l⁻¹ (Table 4.5).

On 21st day VFA concentration was estimated significantly more in T₃ (Cow dung + Kitchen waste) 1.40 g l⁻¹ compared to T₅ (Cow dung + Fruit waste) 1.33 g l⁻¹, T₄ (Cow dung + Maize stalks) 0.85 g l⁻¹, T₂ (Cow dung + Poultry litter) 0.73 g l⁻¹, T₆ (Cow dung alone) 0.47 g l⁻¹ and less in T₁ (Cow dung + Press mud) 0.42 g l⁻¹ (Table 4.5).

On 28th day VFA concentration was estimated significantly more in T₅ (Cow dung + Fruit waste) 1.20 g l⁻¹, compared to T₄ (Cow dung + Maize stalks) 0.80 g l⁻¹, T₆ (Cow dung alone) 0.72 g l⁻¹, T₃ (Cow dung + Kitchen waste) 0.70 g l⁻¹, T₂ (Cow dung + Poultry litter) 0.65 g l⁻¹ and less in T₁ (Cow dung + Press mud) 0.46 g l⁻¹ (Table 4.5).

On 42nd day VFA concentration was estimated significantly more in T₄ (Cow dung + Maize stalks) 2.17 g l⁻¹ compared to T₅ (Cow dung + Fruit waste) 2.08 g l⁻¹, T₂ (Cow dung + Poultry litter) 1.97 g l⁻¹, T₆ (Cow dung alone) 1.91 g l⁻¹, T₃ (Cow dung + Kitchen waste) 1.86 g l⁻¹ and less in T₁ (Cow dung + Press mud) 1.55 g l⁻¹ (Table 4.5).

At the end of the process (on 56th day) the VFA concentration was significantly more in T₄ (Cow dung + Maize stalks) 2.27 g l⁻¹ compared to T₅ (Cow dung + Fruit waste) 2.20 g l⁻¹, T₆ (Cow dung alone) 2.18 g l⁻¹, T₂ (Cow dung + Poultry litter) 2.02 g l⁻¹, T₃ (Cow dung + Kitchen waste) 2.00 g l⁻¹ and less in T₁ (Cow dung + Press mud) 1.98 g l⁻¹ (Table 4.5).

Pham *et al.* (2013) demonstrated that VFA in the cow manure and pig manure was 2.67 and 6.65 g kg⁻¹ respectively.

4.1.5 pH

pH in the substrate was significantly more in T₁ (Cow dung + Press mud) 7.66 compared to T₆ (Cow dung alone) 7.37, T₄ (Cow dung + Maize stalks) 7.01, T₂ (Cow

dung + Poultry litter) 6.87, T₅ (Cow dung + Fruit waste) 6.34 and in T₃ (Cow dung + Kitchen waste) 5.77 (Table 4.1).

In the above result pH in the cow dung was similar to the observation of Abubakar *et al.* (2012) who investigated the effectiveness of cow dung for biogas production and the pH of cow dung was in between 7.1 and 7.4 and during the anaerobic digestion there were variations between 6.5 to 7.6.

In the above result pH in the vegetable wastes was 5.77 and similar to the result observed by Velmurugan *et al.* (2011). He demonstrated that vegetable wastes (Banana stem, Cabbage and Ladies finger) were an-aerobically digested and the vegetable wastes were fed with an average pH of 5.75.

In the inoculum mixture (on zero day), the pH it was significantly more in T₁ (Cow dung + Press mud) 7.60 compared to T₆ (Cow dung alone) 7.54 which was on par and others T₄ (Cow dung + Maize stalks) 7.11, T₂ (Cow dung + Poultry litter) 6.94, T₅ (Cow dung + Fruit waste) 6.47 and in T₃ (Cow dung + Kitchen waste) 5.81 (Table 4.6).

On 7th day, pH was significantly more in T₁ (Cow dung + Press mud) 7.53 compared to T₆ (Cow dung alone) 7.30, T₄ (Cow dung + Maize stalks) 6.92, T₂ (Cow dung + Poultry litter) 6.67, T₅ (Cow dung + Fruit waste) 6.28 and less in T₃ (Cow dung + Kitchen waste) 5.67 (Table 4.6).

On 14th day, pH was significantly more in T₁ (Cow dung + Press mud) 7.43 compared to T₆ (Cow dung alone) 7.18, T₄ (Cow dung + Maize stalks) 6.72, T₂ (Cow dung + Poultry litter) 6.60, T₅ (Cow dung + Fruit waste) 6.08 and less in T₃ (Cow dung + Kitchen waste) 5.57 (Table 4.6).

On 21st day, pH was significantly more in T₆ (Cow dung alone) 7.30 compared to T₁ (Cow dung + Press mud) 7.24, T₄ (Cow dung + Maize stalks) 7.01, T₂ (Cow dung + Poultry litter) 6.87, T₅ (Cow dung + Fruit waste) 6.37 and less in T₃ (Cow dung + Kitchen waste) 5.81 (Table 4.6).

On 28th day, pH was significantly more in T₁ (Cow dung + Press mud) 7.38 and T₂ (Cow dung + Poultry litter) 7.38 compared to T₆ (Cow dung alone) 7.01, T₄ (Cow dung + Maize stalks) 5.50, T₃ (Cow dung + Kitchen waste) 4.80 and less in T₅ (Cow dung + Fruit waste) 4.19 (Table 4.6).

In the above result, pH in the poultry litter was 7.38 and it was similar to the observation of Espinosa-Solares *et al.* (2010) who conducted experiments on the production of biogas from poultry litter. The pH variations in the poultry litter were in between 7.3 and 7.7. First day pH was 7.5 in the poultry litter and later it was decreased to 7.3 finally the pH was 7.6.

On 42nd day, pH was significantly more in T₁ (Cow dung + Press mud) 7.04 compared to T₃ (Cow dung + Kitchen waste) 6.65, T₆ (Cow dung alone) 6.54, T₂ (Cow dung + Poultry litter) 5.47, T₅ (Cow dung + Fruit waste) 4.21 and less in T₄ (Cow dung + Maize stalks) 4.05 (Table 4.6).

pH at the end of the process (on 56th day) was significantly more in T₆ (Cow dung alone) 6.82 compared to T₁ (Cow dung + Press mud) 6.75, T₂ (Cow dung + Poultry litter) 5.62, T₃ (Cow dung + Kitchen waste) 4.83, T₄ (Cow dung + Maize stalks) 4.70 and less in T₅ (Cow dung + Fruit waste) 3.67 (Table 4.6).

4.1.6 Nitrogen (N), Phosphorus (P) and Potassium (K)

4.1.6.1 Nitrogen

The nitrogen per cent in the substrates was significantly more in T₂ (Cow dung + Poultry litter) 2.62 per cent compared to T₃ (Cow dung + Kitchen waste) 2.40 per cent, T₆ (Cow dung alone) 2.09 per cent, T₅ (Cow dung + Fruit waste) 1.85 per cent, T₁ (Cow dung + Press mud) 1.75 per cent and less in T₄ (Cow dung + Maize stalks) 1.34 per cent (Table 4.1).

N per cent in cow dung (2.03 %) observed in the present experiment is similar to the values obtained by Wani *et al.* (2013) who had reported that the N per cent in the cow dung was 1.97 per cent.

Nitrogen per cent was comparatively less in cow dung than in the poultry litter and the obtained results were similar to that of Uzodinma and Ofoefule (2009) who carried out an investigation on the production of biogas, a low natural gas, from equal blending of field grass with some animal wastes which included cow dung and poultry dung. The nitrogen per cent in the substrates was 1.50 per cent and 1.92 per cent.

The significantly more N per cent in cow dung compared to press mud from the present work is in line with the results presented by Jeyabal *et al.* (2001) who studied the composition of nutrients like N, P and K in cow dung, press mud and in the biogas digested slurry. N (1.65 %) and K (0.81 %) are more in the cow dung than in the press mud while P was significantly more in press mud (1.20 %) compared to cow dung (0.70 %).

In the inoculum mixture (on zero day), the N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.56 per cent compared to T₃ (Cow dung + Kitchen waste) 2.36 per cent, T₆ (Cow dung alone) 2.01 per cent, T₅ (Cow dung + Fruit waste) 1.78 per cent, T₁ (Cow dung + Press mud) 1.68 per cent and less in T₄ (Cow dung + Maize stalks) 1.28 per cent (Table 4.7).

On 7th day, N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.64 per cent compared to T₃ (Cow dung + Kitchen waste) 2.43 per cent, T₆ (Cow dung alone) 2.21 per cent, T₅ (Cow dung + Fruit waste) 1.88 per cent, T₁ (Cow dung + Press mud) 1.73 per cent and less in T₄ (Cow dung + Maize stalks) 1.37 per cent (Table 4.7).

On 14th day, N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.77 per cent compared to T₃ (Cow dung + Kitchen waste) 2.44 per cent, T₆ (Cow dung alone) 2.18 per cent, T₅ (Cow dung + Fruit waste) 1.89 per cent, T₁ (Cow dung + Press mud) 1.79 per cent and less in T₄ (Cow dung + Maize stalks) 1.43 per cent (Table 4.7).

On 21st day, N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.80 per cent compared to T₃ (Cow dung + Kitchen waste) 2.50 per cent, T₆ (Cow dung alone) 2.20 per cent, T₅ (Cow dung + Fruit waste) 1.91 per cent, T₁ (Cow dung + Press mud) 1.82 per cent and less in T₄ (Cow dung + Maize stalks) 1.45 per cent (Table 4.7).

On 28th day, N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.52 per cent compared to T₃ (Cow dung + Kitchen waste) 2.43 per cent, T₆ (Cow dung alone) 2.07 per cent, T₅ (Cow dung + Fruit waste) 1.78 per cent, T₁ (Cow dung + Press mud) 1.62 per cent and less in T₄ (Cow dung + Maize stalks) 1.33 per cent (Table 4.7).

On 42nd day, N per cent was significantly more in T₂ (Cow dung + Poultry litter) 2.52 per cent compared to T₃ (Cow dung + Kitchen waste) 2.32 per cent, T₆ (Cow dung alone) 1.88 per cent, T₅ (Cow dung + Fruit waste) 1.56 per cent, T₁ (Cow dung + Press mud) 1.53 per cent and less in T₄ (Cow dung + Maize stalks) 1.03 per cent (Table 4.7).

By the end of the process (on 56th day), N per cent in the digesters was significantly reduced and more reduction in T₂ (Cow dung + Poultry litter) 2.19 per cent compared to T₃ (Cow dung + Kitchen waste) 2.04 per cent, T₅ (Cow dung + Fruit waste) 1.83 per cent, T₆ (Cow dung alone) 1.69 per cent, T₁ (Cow dung + Press mud) 1.50 per cent and less in T₄ (Cow dung + Maize stalks) 0.99 per cent. The reduction in N per cent it was significantly more in T₄ (Cow dung + Maize stalks) 26.12 per cent and less reduction in T₁ (Cow dung + Press mud) 14.29 per cent.

4.1.6.2 Phosphorus

The phosphorus per cent in the substrates was significantly more in T₁ (Cow dung + Press mud) 1.87 per cent compared to T₂ (Cow dung + Poultry litter) 1.54 per cent, T₅ (Cow dung + Fruit waste) 1.44 per cent, T₃ (Cow dung + Kitchen waste) 1.35 per cent, T₆ (Cow dung alone) 1.16 per cent and less in T₄ (Cow dung + Maize stalks) 0.70 per cent (Table 4.1).

In the above results, phosphorus per cent in the press mud was close to that reported by Ramana *et al.* (1999) who studied the nutrient status of press mud and

explained that press mud contain 20-30 per cent organic matter, 0.94-1.3 per cent nitrogen, 1.66-2.74 per cent phosphorus and 0.46- 1.3 per cent potassium.

In the inoculum mixture (on zero day), the P per cent was significantly more in T₁ (Cow dung + Press mud) 1.85 per cent compared to T₂ (Cow dung + Poultry litter) 1.51 per cent, T₅ (Cow dung + Fruit waste) 1.43 per cent, T₃ (Cow dung + Kitchen waste) 1.33 per cent, T₆ (Cow dung alone) 1.15 per cent and less in T₄ (Cow dung + Maize stalks) 0.68 per cent (Table 4.8).

On 7th day, P per cent was significantly more in T₁ (Cow dung + Press mud) 1.81 per cent compared to T₂ (Cow dung + Poultry litter) 1.44 per cent, T₅ (Cow dung + Fruit waste) 1.39 per cent, T₃ (Cow dung + Kitchen waste) 1.30 per cent, T₆ (Cow dung alone) 1.08 per cent and less in T₄ (Cow dung + Maize stalks) 0.65 per cent (Table 4.8).

On 14th day, P per cent was significantly more in T₁ (Cow dung + Press mud) 1.78 per cent compared to T₂ (Cow dung + Poultry litter) 1.42 per cent, T₅ (Cow dung + Fruit waste) 1.37 per cent, T₃ (Cow dung + Kitchen waste) 1.26 per cent, T₆ (Cow dung alone) 1.06 per cent and less in T₄ (Cow dung + Maize stalks) 0.64 per cent (Table 4.8).

On 21st day, P per cent was significantly more in T₁ (Cow dung + Press mud) 1.78 per cent compared to T₂ (Cow dung + Poultry litter) 1.42 per cent, T₅ (Cow dung + Fruit waste) 1.37 per cent, T₃ (Cow dung + Kitchen waste) 1.26 per cent, T₆ (Cow dung alone) 1.06 per cent and less in T₄ (Cow dung + Maize stalks) 0.64 per cent (Table 4.8).

On 28th day, P per cent was significantly more in T₁ (Cow dung + Press mud) 1.65 per cent compared to T₂ (Cow dung + Poultry litter) 1.37 per cent, T₅ (Cow dung + Fruit waste) 1.31 per cent, T₃ (Cow dung + Kitchen waste) 1.15 per cent, T₆ (Cow dung alone) 0.98 per cent and less in T₄ (Cow dung + Maize stalks) 0.58 per cent (Table 4.8).

On 42nd day, P per cent was significantly more in T₁ (Cow dung + Press mud) 1.61 per cent compared to T₂ (Cow dung + Poultry litter) 1.30 per cent, T₅ (Cow dung + Fruit waste) 1.22 per cent, T₃ (Cow dung + Kitchen waste) 1.12 per cent, T₆ (Cow dung alone) 0.95 per cent and less in T₄ (Cow dung + Maize stalks) 0.52 per cent (Table 4.8).

At the end of the process (on 56th day), P per cent in the digester was reduced and in T₁ (Cow dung + Press mud) 1.55 per cent compared to T₂ (Cow dung + Poultry litter) 1.25 per cent, T₅ (Cow dung + Fruit waste) 1.14 per cent, T₃ (Cow dung + Kitchen waste) 1.08 per cent, T₆ (Cow dung alone) 0.94 per cent and less in T₄ (Cow dung + Maize stalks) 0.44 per cent. The reduction of P per cent was significantly more in T₄ (Cow dung + Maize stalks) 37.15 per cent and less reduction in T₁ (Cow dung + Press mud) 17.12 per cent (Table 4.8).

4.1.6.3 Potassium

The potassium per cent in the substrates was significantly more in T₂ (Cow dung + Poultry litter) 1.25 per cent compared to T₃ (Cow dung + Kitchen waste) 1.13 per cent, T₅ (Cow dung + Fruit waste) 0.82 per cent, T₁ (Cow dung + Press mud) 0.78 per cent, T₆ (Cow dung alone) 0.66 per cent and less in T₄ (Cow dung + Maize stalks) 0.50 per cent (Table 4.1).

In the above result K per cent in the press mud was similar to observations of Patil *et al.* (2013) who studied the isolation and enrichment of sugar press mud (spm) adapted microorganisms for production of biofertilizer by using sugar press mud. The composition of press mud was studied and it contained 0.22 per cent nitrogen, 0.2 per cent phosphorus and 1.27 per cent potassium.

In the results obtained K per cent it was significantly more in kitchen wastes compared to cow dung and this was justified from the observation of Wani *et al.* (2013) who worked on the nutrient content and different physio-chemical parameters in garden waste, kitchen waste and cow dung. He explained that K content was significantly more in kitchen wastes (1.01 %) compared to cow dung (0.88 %) and garden wastes (0.60 %).

In the results obtained K per cent in the poultry litter was similar to the observations of Gosh *et al.* (2004) who conducted field experiments on deep vertisols of Bhopal, India to evaluate the manurial potential of three organic manures: FarmYard Manure (FYM), Poultry Manure (PM) and PhosphoCompost (PC). From his experiments he found that poultry manure contains 2.14 per cent N, 1.09 per cent P and 1.3 per cent K.

In the inoculum mixture (on zero day), the K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.24 per cent compared to T₃ (Cow dung + Kitchen waste) 1.11 per cent, T₅ (Cow dung + Fruit waste) 0.81 per cent, T₁ (Cow dung + Press mud) 0.75 per cent, T₆ (Cow dung alone) 0.62 per cent and less in T₄ (Cow dung + Maize stalks) 0.47 per cent (Table 4.9).

On 7th day, K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.26 per cent compared to T₃ (Cow dung + Kitchen waste) 1.16 per cent, T₅ (Cow dung + Fruit waste) 0.85 per cent, T₁ (Cow dung + Press mud) 0.78 per cent, T₆ (Cow dung alone) 0.68 per cent and less in T₄ (Cow dung + Maize stalks) 0.51 per cent (Table 4.9).

On 14th day, K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.36 per cent compared to T₃ (Cow dung + Kitchen waste) 1.20 per cent, T₅ (Cow dung + Fruit waste) 0.89 per cent, T₁ (Cow dung + Press mud) 0.81 per cent, T₆ (Cow dung alone) 0.71 per cent and less in T₄ (Cow dung + Maize stalks) 0.58 per cent (Table 4.9).

On 21st day, K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.42 per cent compared to T₃ (Cow dung + Kitchen waste) 1.28 per cent, T₅ (Cow dung + Fruit waste) 1.05 per cent, T₁ (Cow dung + Press mud) 0.95 per cent, T₆ (Cow dung alone) 0.85 per cent and less in T₄ (Cow dung + Maize stalks) 0.66 per cent (Table 4.9).

On 28th day, K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.52 per cent compared to T₃ (Cow dung + Kitchen waste) 1.30 per cent, T₅ (Cow dung + Fruit waste) 1.12 per cent, T₁ (Cow dung + Press mud) 0.98 per cent, T₆ (Cow dung alone) 0.88 per cent and less in T₄ (Cow dung + Maize stalks) 0.71 per cent (Table 4.9).

On 42nd day, K per cent was significantly more in T₂ (Cow dung + Poultry litter) 1.58 per cent compared to T₃ (Cow dung + Kitchen waste) 1.32 per cent, T₅ (Cow dung + Fruit waste) 1.16 per cent, T₁ (Cow dung + Press mud) 1.13 per cent, T₆ (Cow dung alone) 0.94 per cent and less in T₄ (Cow dung + Maize stalks) 0.80 per cent (Table 4.9).

In the above results, K per cent was significantly more in cow dung compared to maize stalks and this was justified from the work of Kolar *et al.* (2010) who used mixture of cattle slurry, maize silage and grass haylage for biogas production and showed that the cattle slurry was with more nutrients (N, P, K) 2.4 per cent, 1.3 per cent, 5.3 per cent compared to maize silage 0.1 per cent, 0.2 per cent, 1.4 per cent respectively.

At the end of the process (on 56th day) K per cent in the digester was increased and in T₂ (Cow dung + Poultry litter) 1.60 per cent compared to T₃ (Cow dung + Kitchen waste) 1.37 per cent, T₁ (Cow dung + Press mud) 1.27 per cent, T₅ (Cow dung + Fruit waste) 1.24 per cent, T₆ (Cow dung alone) 0.96 per cent and less in T₄ (Cow dung + Maize stalks) 0.84 per cent. The increase in K per cent was significantly more in T₁ (Cow dung + Press mud) 40.95 per cent and less increase in T₃ (Cow dung + Kitchen waste) 19.71 per cent (Table 4.9).

In the above results N per cent and P per cent in the process of anaerobic digestion were reduced while K per cent was increased and this result was justified from the observations of Singh *et al.* (2009) who conducted chemical tests on the poultry slurry and showed that the concentration of potassium was increased and the concentration of phosphorus and nitrogen were decreased on anaerobic digestion. The percentage of N in the raw poultry waste was 2.01. It decreased gradually to 2.00 during the first two weeks of fermentation and then to an average of 1.79 percent finally. The P content of the raw material was reported to be 6.66 percent. It was decreased to 4.37 percent after two weeks digestion and finally 5.85 percent was recorded as the average

value. As for K, it increased from 3.45 percent to 4.15 percent after two weeks digestion process with final average value of 5.51 percent.

4.1.7 Organic carbon per cent

The organic carbon per cent in the substrates was significantly more in T₆ (Cow dung alone) 52.15 per cent compared to T₅ (Cow dung + Fruit waste) 50.78 per cent, T₃ (Cow dung + Kitchen waste) 50.22 per cent, T₂ (Cow dung + Poultry litter) 48.52 per cent, T₁ (Cow dung + Press mud) 46.97 per cent and less in T₄ (Cow dung + Maize stalks) 36.18 per cent (Table 4.1).

In the above result the organic carbon per cent in the cow dung was similar to the results reported by Yadav *et al.* (2013) who conducted experiments and showed results that indicate cow dung and biogas plant slurry can be used as a raw material in vermicomposting. The organic carbon content was 425 to 550 g kg⁻¹ for cow dung and 400 to 470 g kg⁻¹ for biogas plant slurry.

Suthar (2010) demonstrated that the organic carbon in the cow dung was 329.8 gkg⁻¹, in biogas slurry it was 359.5 g kg⁻¹ and in vegetable wastes was 390.1 g kg⁻¹. Bernal *et al.* (2009) worked on composting of animal manures and showed that the organic carbon content in liquid poultry manure was 11 – 112 g kg⁻¹ and in solid poultry manure was 103 – 597 g kg⁻¹. Bouallagui *et al.* (2004) explained the laboratory scale experiments of fruit and vegetable wastes anaerobic digestion. The organic carbon content of the substrate was found to be 50.9 per cent on an average. Jeyabal *et al.* (2001) studied the composition of organic carbon per cent in cow dung and press mud. The organic carbon per cent it was significantly more in cow dung (47.3 %) compared to that in the press mud (44 %). Where as in the biogas digested slurry the organic carbon per cent was 27.3 per cent.

The organic carbon per cent in the inoculum mixture (on zero day) was significantly more in T₆ (Cow dung alone) 50.74 per cent compared to T₅ (Cow dung + Fruit waste) 50.23 per cent, T₃ (Cow dung + Kitchen waste) 49.05 per cent, T₂ (Cow dung + Poultry litter) 46.86 per cent, T₁ (Cow dung + Press mud) 46.04 per cent and less in T₄ (Cow dung + Maize stalks) 35.12 per cent (Table 4.10).

The organic carbon per cent on 7th day was significantly more in T₆ (Cow dung alone) 49.81 per cent compared to, T₅ (Cow dung + Fruit waste) 49.10 T₃ (Cow dung + Kitchen waste) 47.78 per cent, T₂ (Cow dung + Poultry litter) 45.78 per cent, T₁ (Cow dung + Press mud) 45.24 per cent and less in T₄ (Cow dung + Maize stalks) 34.18 per cent (Table 4.10).

The organic carbon per cent on 14th day was significantly more in T₆ (Cow dung alone) 49.30 per cent compared to, T₅ (Cow dung + Fruit waste) 48.22 per cent T₃ (Cow dung + Kitchen waste) 46.84 per cent, T₂ (Cow dung + Poultry litter) 45.24 per cent, T₁ (Cow dung + Press mud) 44.66 per cent and less in T₄ (Cow dung + Maize stalks) 33.58 per cent (Table 4.10).

The organic carbon per cent on 21st day was significantly more in T₅ (Cow dung + Fruit waste) 46.25 per cent compared to, T₆ (Cow dung alone) 45.29 per cent T₃ (Cow dung + Kitchen waste) 45.00 per cent, T₂ (Cow dung + Poultry litter) 43.33 per cent, T₁ (Cow dung + Press mud) 42.91 per cent and less in T₄ (Cow dung + Maize stalks) 31.13 per cent (Table 4.10).

The organic carbon per cent on 28th day was significantly more in T₅ (Cow dung + Fruit waste) 47.54 per cent compared to, T₆ (Cow dung alone) 46.17 per cent T₃ (Cow dung + Kitchen waste) 46.06 per cent, T₂ (Cow dung + Poultry litter) 44.33 per cent, T₁ (Cow dung + Press mud) 43.53 per cent and less in T₄ (Cow dung + Maize stalks) 32.30 per cent (Table 4.10).

The organic carbon per cent on 42nd day was significantly more in T₅ (Cow dung + Fruit waste) 45.66 per cent compared to, T₆ (Cow dung alone) 42.96 per cent, T₂ (Cow dung + Poultry litter) 42.66 per cent, T₃ (Cow dung + Kitchen waste) 42.00 per cent, T₁ (Cow dung + Press mud) 41.94 per cent and less in T₄ (Cow dung + Maize stalks) 30.69 per cent (Table 4.10).

At the end of the process (on 56th day) the reduction in organic carbon per cent was observed and reduction was significantly more in T₆ (Cow dung alone) 30 per cent (52.15 % in the first day to 36.51 % at the end of the process) and less reduction was observed in T₁ (Cow dung + Press mud) 13.44 per cent (46.97 % in the first day to 40.66 % at the end of the process) (Table 4.10).

The reduction in organic carbon per cent was observed in all the treatments at the end of the process. The reduction was significantly more in T₁ (Cow dung + Press mud) 40.66 per cent, T₂ (Cow dung + Poultry litter) 40.50 per cent, T₃ (Cow dung + Kitchen waste) 36.74 per cent, (Cow dung + Maize stalks) 30.34 per cent, T₅ (Cow dung + Fruit waste) 39.49 per cent and in T₆ (Cow dung alone) 36.51 per cent.

4.1.8 Biological Oxygen demand (BOD) and Chemical Oxygen Demand (COD)

4.1.8.1 Biological Oxygen Demand (BOD)

Biological oxygen demand in the substrate was significantly more in T₆ (Cow dung alone) 167.18 mg l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 144.62 mg l⁻¹, T₂ (Cow dung + Poultry litter) 129.69 mg l⁻¹, T₁ (Cow dung + Press mud) 123.70 mg l⁻¹,

T₄ (Cow dung + Maize stalks) 112.28 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 79.58 mg l⁻¹ (Table 4.1).

BOD in the inoculum mixture (on zero day) was significantly more in T₆ (Cow dung alone) 151.75 mg l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 134.85 mg l⁻¹, T₂ (Cow dung + Poultry litter) 118.00 mg l⁻¹, T₄ (Cow dung + Maize stalks) 106.88 mg l⁻¹, T₁ (Cow dung + Press mud) 101.00 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 67.25 mg l⁻¹ (Table 4.11).

BOD on 7th day was significantly more in T₆ (Cow dung alone) 139.51 mg l⁻¹, compared to T₃ (Cow dung + Kitchen waste) 124.057 mg l⁻¹, T₂ (Cow dung + Poultry litter) 104.00 mg l⁻¹, T₄ (Cow dung + Maize stalks) 95.02 mg l⁻¹, T₁ (Cow dung + Press mud) 94.33 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 60.64 mg l⁻¹ (Table 4.11).

BOD on 14th day was significantly more in T₆ (Cow dung alone) 132.43 mg l⁻¹, compared to T₃ (Cow dung + Kitchen waste) 121.47 mg l⁻¹, T₂ (Cow dung + Poultry litter) 103.15 mg l⁻¹, T₄ (Cow dung + Maize stalks) 96.76 mg l⁻¹, T₁ (Cow dung + Press mud) 90.67 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 51.32 mg l⁻¹ (Table 4.11).

BOD on 21st day was significantly more in T₆ (Cow dung alone) 117.40 mg l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 107.64 mg l⁻¹, T₂ (Cow dung + Poultry litter) 91.28 mg l⁻¹, T₄ (Cow dung + Maize stalks) 90.71 mg l⁻¹, T₁ (Cow dung + Press mud) 80.14 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 44.00 mg l⁻¹ (Table 4.11).

BOD on 28th day was significantly more in T₆ (Cow dung alone) 103.67 mg l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 95.01 mg l⁻¹, T₄ (Cow dung + Maize stalks) 84.78 mg l⁻¹, T₁ (Cow dung + Press mud) 76.83 mg l⁻¹, T₂ (Cow dung + Poultry litter) 76.26 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 39.87 mg l⁻¹ (Table 4.11).

BOD on 42nd day was significantly more in T₆ (Cow dung alone) 92.83 mg l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 83.55 mg l⁻¹, T₄ (Cow dung + Maize stalks) 82.98 mg l⁻¹, T₁ (Cow dung + Press mud) 71.90 mg l⁻¹, T₂ (Cow dung + Poultry litter) 74.74 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 35.70 mg l⁻¹ (Table 4.11).

At the end of the process (on 56th day) the reduction in BOD was observed and significantly more reduction was in T₅ (Cow dung + Fruit waste) 58.74 per cent compared to T₂ (Cow dung + Poultry litter) 52.20 per cent T₆ (Cow dung alone) 51.03 per cent, T₃ (Cow dung + Kitchen waste) 50.30 per cent, T₁ (Cow dung + Press mud) 45.57 per cent and less reduction in T₄ (Cow dung + Maize stalks) 27.68 per cent (Table 4.11).

The above results were similar to that of Bhumesh Singh and sai (2011) who studied on biogas generation from dairy effluent and explained that BOD and COD reduced to the extent of 50 per cent by the end of the process.

4.1.8.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand in the substrate was significantly more in T₆ (Cow dung alone) 148.61 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 138.28 g l⁻¹, T₄ (Cow dung + Maize stalks) 130.72 g l⁻¹, T₁ (Cow dung + Press mud) 123.02 g l⁻¹, T₅ (Cow dung + Fruit waste) 115.11 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 109.55 g l⁻¹ (Table 4.1).

In the above results, the COD value in the cow dung was similar to that of Li and jha (2014) who studied the characteristics of semi-dry anaerobic digestion of cow dung and pig manure mixture under psychrophilic condition. The Chemical Oxygen Demand (COD) in the substrates was estimated. In cow dung the COD was 150.54 g l⁻¹ and in pig manure 163.63 g l⁻¹.

Gonzalez *et al.* (2013) did work on the thermo-chemical pre-treatment to solubilize and improve anaerobic biodegradability of press mud. COD was 86.20 g l⁻¹.

Espinosa-Solares *et al.* (2010) conducted experiments on the short term effects of temperature changes in a pilot plant for the production of biogas from poultry litter. The average influent COD was 52 g l⁻¹.

COD in the inoculum mixture (on zero day) was significantly more in T₆ (Cow dung alone) 131.41 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 130.53 g l⁻¹, T₄ (Cow dung + Maize stalks) 125.78 g l⁻¹, T₁ (Cow dung + Press mud) 110.24 g l⁻¹, T₅ (Cow dung + Fruit waste) 105.44 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 101.35 g l⁻¹ (Table 4.12).

COD on 7th day was significantly more in T₆ (Cow dung alone) 138.24 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 133.81 g l⁻¹, T₄ (Cow dung + Maize stalks) 127.49 g l⁻¹, T₁ (Cow dung + Press mud) 111.85 g l⁻¹, T₅ (Cow dung + Fruit waste) 109.54 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 94.81 g l⁻¹ (Table 4.12).

COD on 14th day was significantly more in T₃ (Cow dung + Kitchen waste) 128.28 g l⁻¹ compared to T₆ (Cow dung alone) 124.74 g l⁻¹, T₄ (Cow dung + Maize stalks) 123.58 g l⁻¹, T₁ (Cow dung + Press mud) 107.82 g l⁻¹, T₅ (Cow dung + Fruit waste) 103.93 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 87.27 g l⁻¹ (Table 4.12).

COD on 21st day was significantly more in T₄ (Cow dung + Maize stalks) 118.78 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 113.12 g l⁻¹, T₆ (Cow dung

alone) 102.38 g l⁻¹, T₁ (Cow dung + Press mud) 101.59 g l⁻¹, T₅ (Cow dung + Fruit waste) 97.84 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 72.01 g l⁻¹ (Table 4.12).

COD on 28th day was significantly more in T₄ (Cow dung + Maize stalks) 113.59 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 104.71 g l⁻¹, T₅ (Cow dung + Fruit waste) 95.47 g l⁻¹, T₁ (Cow dung + Press mud) 92.59 g l⁻¹, T₆ (Cow dung alone) 84.57 g l⁻¹, and less in T₂ (Cow dung + Poultry litter) 62.52 g l⁻¹ (Table 4.12).

COD on 42nd day was significantly more in T₄ (Cow dung + Maize stalks) 109.53 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 90.99 g l⁻¹, T₅ (Cow dung + Fruit waste) 90.42 g l⁻¹, T₁ (Cow dung + Press mud) 85.14 g l⁻¹, T₆ (Cow dung alone) 78.74 g l⁻¹, and less in T₂ (Cow dung + Poultry litter) 58.57 g l⁻¹ (Table 4.12).

COD at the end of the process was significantly more in T₄ (Cow dung + Maize stalks) 106.53 g l⁻¹ compared to T₃ (Cow dung + Kitchen waste) 104.71 g l⁻¹, T₅ (Cow dung + Fruit waste) 95.47 g l⁻¹, T₁ (Cow dung + Press mud) 92.59 g l⁻¹, T₆ (Cow dung alone) 84.57 g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 62.52 g l⁻¹ (Table 4.12).

At the end of the process (on 56th day) decrease in COD was observed and more decrease was in T₆ (Cow dung alone) 52.15 per cent compared to T₂ (Cow dung + Poultry litter) 49.88 per cent, T₁ (Cow dung + Press mud) 41.55 per cent, T₃ (Cow dung + Kitchen waste) 40.26 per cent, T₅ (Cow dung + Fruit waste) 30.04 per cent and less reduction in T₄ (Cow dung + Maize stalks) 18.77 per cent (Table 4.12).

By the end of the process the COD reduction in cow dung was 52.15 per cent this result was almost similar to the result of Abubakar and Ismail (2012) who investigated the effectiveness of cow dung for biogas production and explained that at the end of the process cow dung digestion reaches 48.5 per cent COD reduction.

4.1.9 Electrical conductivity (EC)

The EC in the substrate was significantly more in T₁ (Cow dung + Press mud) 5.42 dsm⁻¹ compared to T₅ (Cow dung + Fruit waste) 4.03 dsm⁻¹, T₂ (Cow dung + Poultry litter) 3.93 dsm⁻¹, T₄ (Cow dung + Maize stalks) 3.73 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.54 dsm⁻¹ and less in T₆ (Cow dung alone) 2.2 dsm⁻¹ (Table 4.1).

The EC in the cow dung was similar to that of the results showed by Yadav *et al.* (2013) who indicated that the cow dung has an EC of 1.2 to 2.2 dsm⁻¹.

In the inoculum mixture (on zero day), the EC was significantly more in T₁ (Cow dung + Press mud) 5.04 dsm⁻¹ as compared to T₅ (Cow dung + Fruit waste) 3.8 dsm⁻¹, T₂ (Cow dung + Poultry litter) 3.52 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.42 dsm⁻¹, T₄ (Cow dung + Maize stalks) 2.62 dsm⁻¹ and less in T₆ (Cow dung alone) 2.07 dsm⁻¹ (Table 4.13).

On 7th day EC was significantly more in T₁ (Cow dung + Press mud) 4.28 dsm⁻¹ compared to T₅ (Cow dung + Fruit waste) 3.57 dsm⁻¹, T₂ (Cow dung + Poultry litter) 3.28 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.23 dsm⁻¹, T₄ (Cow dung + Maize stalks) 2.42 dsm⁻¹ and less in T₆ (Cow dung alone) 1.79 dsm⁻¹ (Table 4.13).

On 14th day EC was significantly more in T₅ (Cow dung + Fruit waste) 3.62 dsm⁻¹ compared to T₁ (Cow dung + Press mud) 3.36 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.23 dsm⁻¹, T₂ (Cow dung + Poultry litter) 3.17 dsm⁻¹, T₄ (Cow dung + Maize stalks) 2.20 dsm⁻¹ and less in T₆ (Cow dung alone) 1.86 dsm⁻¹ (Table 4.13).

On 21st day EC was significantly more in T₅ (Cow dung + Fruit waste) 3.51 dsm⁻¹ compared to T₁ (Cow dung + Press mud) 3.27 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.12 dsm⁻¹, T₂ (Cow dung + Poultry litter) 3.06 dsm⁻¹, T₄ (Cow dung + Maize stalks) 2.11 dsm⁻¹ and less in T₆ (Cow dung alone) 1.71 dsm⁻¹ (Table 4.13).

On 28th day EC was significantly more in T₅ (Cow dung + Fruit waste) 3.51 dsm⁻¹ compared to T₁ (Cow dung + Press mud) 3.21 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 3.03 dsm⁻¹, T₂ (Cow dung + Poultry litter) 2.83 dsm⁻¹, T₄ (Cow dung + Maize stalks) 2.01 dsm⁻¹ and less in T₆ (Cow dung alone) 1.66 dsm⁻¹ (Table 4.13).

On 42nd day EC was significantly more in T₁ (Cow dung + Press mud) 3.02 dsm⁻¹ compared to T₃ (Cow dung + Kitchen waste) 2.85 dsm⁻¹, T₂ (Cow dung + Poultry litter) 2.67 dsm⁻¹, T₅ (Cow dung + Fruit waste) 2.47 dsm⁻¹, T₄ (Cow dung + Maize stalks) 1.85 dsm⁻¹ and less in T₆ (Cow dung alone) 1.52 dsm⁻¹ (Table 4.13).

At the end of the process (on 56th day), EC was significantly more in T₁ (Cow dung + Press mud) 2.80 dsm⁻¹ compared to T₂ (Cow dung + Poultry litter) 2.21 dsm⁻¹, T₃ (Cow dung + Kitchen waste) 1.90 dsm⁻¹, T₅ (Cow dung + Fruit waste) 1.78 dsm⁻¹, T₆ (Cow dung alone) 1.53 dsm⁻¹ and T₄ (Cow dung + Maize stalks) 1.23 dsm⁻¹ (Table 4.13).

4.1.10 Methane percentage in the evolved biogas

Methane (CH₄) per cent was estimated from the biogas evolved from all the six treatments. Significantly highest CH₄ per cent was obtained in T₄ (Cow dung + Maize stalks) 52.51 per cent which was on par with T₂ (Cow dung + Poultry litter) 52.38 per cent and T₁ (Cow dung + Press mud) 52.08 per cent and comparatively less CH₄ per cent was obtained in T₆ (Cow dung alone) 50.44 per cent, T₅ (Cow dung + Fruit waste) 49.30 per cent and lowest in T₃ (Cow dung + Kitchen waste) 47.95 per cent (Table 4.14).

Carbon dioxide (CO₂) per cent was significantly more in T₅ (Cow dung + Fruit waste) 45.08 per cent which was on par with T₃ (Cow dung + Kitchen waste) 44.96 per cent and comparatively less CO₂ per cent was obtained in T₁ (Cow dung + Press mud)

42.02 per cent, T₂ (Cow dung + Poultry litter) 41.93 per cent, T₄ (Cow dung + Maize stalks) 40.94 per cent and least in T₆ (Cow dung alone) 40.80 per cent (Table 4.14).

Nitrogen (N₂) per cent was significantly more in T₅ (Cow dung + Fruit waste) 0.54 per cent, followed by T₃ (Cow dung + Kitchen waste) 0.45 per cent, T₂ (Cow dung + Poultry litter) 0.33 per cent, T₆ (Cow dung alone) 0.24 per cent, T₄ (Cow dung + Maize stalks) 0.21 per cent and least in T₁ (Cow dung + Press mud) 0.16 per cent.

Narayani *et al.* (2012) explained that the cumulative bio gas production increased, when fruit wastes along with cosubstrates were used. Highest biogas production (405 ml) was obtained for 75 per cent fruit wastes + 25 per cent cow dung sample, where as lowest for the sample 75 per cent fruit wastes + 25 per cent rice bran. Also the methane per cent was highest when the fruit wastes digested with cow dung (80per cent) compared to the other samples.

4.2 ENRICHMENT OF BIOGAS MANURES WITH BENEFICIAL MICROORGANISMS.

4.2.1 Population of beneficial bacteria present in the biogas manures before enrichment.

The population of *Rhizobium* in the biogas manure samples (50 per cent moisture) was significantly more in T₁ (Cow dung + Press mud) 46.0×10^3 CFU g⁻¹ compared to T₆ (Cow dung alone) 34.0×10^3 CFU g⁻¹, T₂ (Cow dung + Poultry litter) 32.0×10^3 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 6.1×10^3 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 5.1×10^3 CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 3.1×10^3 CFU g⁻¹ (Table 4.15).

The population of *Pseudomonas* in the biogas manure samples (50 per cent moisture) was significantly more in T₆ (Cow dung alone) 46.0×10^3 CFU g⁻¹ compared to T₂ (Cow dung + Poultry litter) 44.0×10^3 CFU g⁻¹, T₁ (Cow dung + Press mud) 40.0×10^3 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 4.6×10^3 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 4.0×10^3 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 3.0×10^3 CFU g⁻¹ (Table 4.15).

The population of *Azotobacter* in the biogas manure samples (50 per cent moisture) was significantly more in T₂ (Cow dung + Poultry litter) and T₆ (Cow dung alone) 42.0×10^3 CFU g⁻¹ compared to T₁ (Cow dung + Press mud) 12.0×10^3 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 4.6×10^3 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 3.1×10^3 CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 2.0×10^3 CFU g⁻¹ (Table 4.15).

The population of *Azospirillum* in the biogas manure samples (50 per cent moisture) was significantly more in T₆ (Cow dung alone) 24.0×10^3 CFU g⁻¹ compared to

T₁ (Cow dung + Press mud) 20.0×10³ CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 18.0×10³ CFU g⁻¹, T₂ (Cow dung + Poultry litter) 12.0×10³ CFU g⁻¹, T₅ (Cow dung + Fruit waste) 2.8×10³ CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 2.2×10³ CFU g⁻¹ (Table 4.15).

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. Microbial population in the slurry before enrichment was 80.5×10⁵ bacteria, 124×10³ fungi, 12×10² actinomycetes, 15.3×10³ phosphate solubilise micro organisms and 20.2×10³ free living nitrogen fixers

4.2.2 Viability of the beneficial microorganisms inoculated individually into the unsterilized biogas manure samples.

4.2.2.1 Population of *Rhizobium* after the enrichment.

The population of *Rhizobium* in the Yeast Extract Mannitol (YEM) broth was 3.1×10⁹ CFU ml⁻¹ (Table 4.16).

After the enrichment of the biogas manures with YEM broth, the population of *Rhizobium* on the first day was significantly more in T₆ (Cow dung alone) 4.8×10⁹ CFU g⁻¹ compared to T₁ (Cow dung + Press mud) 3.2×10⁹ CFU g⁻¹, T₂ (Cow dung + Poultry litter) 2.8×10⁹ CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.0×10⁹ CFU g⁻¹, T₅ (Cow dung + Fruit waste) 0.9×10⁹ CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 0.8×10⁹ CFU g⁻¹ (Table 4.18).

There was an increase in the population of *Rhizobium* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₆ (Cow dung alone) 1.2×10⁹ CFU g⁻¹ (4.8×10⁹ CFU g⁻¹, on the first day to 6.1×10⁹ CFU g⁻¹, at the end of fourth week) and less in T₄ (Cow dung + Maize stalks) 0.6×10⁹ CFU g⁻¹ (0.8×10⁹ CFU g⁻¹, on the first day to 1.6×10⁹ CFU g⁻¹, at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T₆ (Cow dung alone) 6.1×10⁹ CFU g⁻¹ at the end of fourth week to 3.0×10⁸ CFU g⁻¹ at the end of sixth week and less reduction in population was observed in T₅ (Cow dung + Fruit waste) 1.9×10⁹ CFU g⁻¹ to 0.8×10⁸ CFU g⁻¹ (Table 4.18).

Finally the viability was significantly more in T₅ (Cow dung + Fruit waste) 0.9×10⁹ CFU g⁻¹, on the first day to 0.5×10⁷ CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₂ (Cow dung + Poultry litter) 2.8×10⁹ CFU g⁻¹ on the first day and 0.8×10⁷ CFU g⁻¹ at the end of tenth week (Table 4.18).

4.2.2.2 Population of *Pseudomonas* after the enrichment.

The population of *Pseudomonas* in Kings B broth was 3.0×10^9 CFU ml⁻¹ (Table 4.16).

After the enrichment of the biogas manures with King's B broth, the population of *Pseudomonas* on the first day was significantly more in T₆ (Cow dung alone) 3.2×10^9 CFU g⁻¹ compared to T₂ (Cow dung + Poultry litter) 2.4×10^9 CFU g⁻¹, T₁ (Cow dung + Press mud) 1.8×10^9 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.6×10^9 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 1.1×10^9 CFU g⁻¹ and less in T₃ (Cow dung + Kitchen waste) 1.0×10^9 CFU g⁻¹ (Table 4.19).

There was an increase in the population of *Pseudomonas* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₃ (Cow dung + Kitchen waste) 2.6×10^9 CFU g⁻¹ (1.0×10^9 CFU g⁻¹ on the first day to 3.6×10^9 CFU g⁻¹ at the end of fourth week) and less in T₂ (Cow dung + Poultry litter) 1.6×10^9 CFU g⁻¹ (2.4×10^9 on the first day to 4.0×10^9 at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 3.7×10^9 CFU g⁻¹ at the end of fourth week to 2.1×10^8 CFU g⁻¹ at the end of sixth week and less reduction was observed in T₂ (Cow dung + Poultry litter) 4.0×10^9 CFU g⁻¹ at the end of fourth week to 2.5×10^8 CFU g⁻¹ at the end of sixth week (Table 4.19).

Finally the viability was significantly more in T₃ (Cow dung + Kitchen waste) 1.0×10^9 CFU g⁻¹ on the first day to 2.0×10^7 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₄ (Cow dung + Maize stalks) 1.6×10^9 CFU g⁻¹ on the first day to 1.4×10^7 CFU g⁻¹ at the end of tenth week (Table 4.19).

4.2.2.3 Population of *Azotobacter* after the enrichment.

The population of *Azotobacter* in *Azotobacter* glucose broth was 2.6×10^9 CFU ml⁻¹ (Table 4.16).

After the enrichment of the biogas manures with *Azotobacter* glucose broth, the population of *Azotobacter* on the first day was significantly more in T₆ (Cow dung alone) 2.8×10^9 CFU g⁻¹ compared to T₁ (Cow dung + Press mud) 2.2×10^9 CFU g⁻¹, T₂ (Cow dung + Poultry litter) 2.0×10^9 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.7×10^9 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 1.2×10^9 CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 1.0×10^9 CFU g⁻¹ (Table 4.20).

There was an increase in the population of *Azotobacter* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₁ (Cow dung + Press mud) 1.6×10^9 CFU g⁻¹ (2.2×10^9 CFU g⁻¹ on the first day to 3.8×10^9 CFU

g^{-1} at the end of third week) and less in T_5 (Cow dung + Fruit waste) $1.2 \times 10^9 \text{ CFU g}^{-1}$ ($1.2 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $1.4 \times 10^9 \text{ CFU g}^{-1}$ at the end of third week). The decrease in population was observed after third week and significantly more reduction in the population was observed in T_4 (Cow dung + Maize stalks) $2.2 \times 10^9 \text{ CFU g}^{-1}$ at the end of third week to $2.5 \times 10^8 \text{ CFU g}^{-1}$ at the end of fourth week and less reduction was in T_1 (Cow dung + Press mud) $3.8 \times 10^9 \text{ CFU g}^{-1}$ to $4.4 \times 10^9 \text{ CFU g}^{-1}$ (Table 4.20).

Finally the viability was significantly more in T_6 (Cow dung alone) $2.8 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $4.1 \times 10^6 \text{ CFU g}^{-1}$ at the end of tenth week and comparatively less viability was observed in T_3 (Cow dung + Kitchen waste) $1.7 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $1.4 \times 10^7 \text{ CFU g}^{-1}$ at the end of tenth week (Table 4.20).

4.2.2.4 Population of *Azospirillum* after the enrichment.

The population of *Azospirillum* in the potato infusion broth was $2.6 \times 10^9 \text{ CFU ml}^{-1}$ (Table 4.16).

After the enrichment of the dried biogas manures with potato infusion broth, the population of *Azospirillum* on the first day was significantly more in T_6 (Cow dung alone) $2.6 \times 10^9 \text{ CFU g}^{-1}$ compared to T_1 (Cow dung + Press mud) $2.4 \times 10^9 \text{ CFU g}^{-1}$, T_2 (Cow dung + Poultry litter) $2.0 \times 10^9 \text{ CFU g}^{-1}$, T_3 (Cow dung + Kitchen waste) $1.4 \times 10^9 \text{ CFU g}^{-1}$, T_4 (Cow dung + Maize stalks) $1.0 \times 10^9 \text{ CFU g}^{-1}$ and less in T_5 (Cow dung + Fruit waste) $0.6 \times 10^9 \text{ CFU g}^{-1}$ (Table 4.21).

There was an increase in the population of *Azospirillum* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T_5 (Cow dung + Fruit waste) $1.7 \times 10^9 \text{ CFU g}^{-1}$ ($0.6 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $2.3 \times 10^9 \text{ CFU g}^{-1}$ at the end of fourth week) and less in T_4 (Cow dung + Maize stalks) $1.1 \times 10^9 \text{ CFU g}^{-1}$ ($1.0 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $2.1 \times 10^9 \text{ CFU g}^{-1}$ at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T_2 (Cow dung + Poultry litter) $3.4 \times 10^9 \text{ CFU g}^{-1}$ at the end of fourth week to $2.0 \times 10^8 \text{ CFU g}^{-1}$ at the end of sixth week and less reduction was observed in T_4 (Cow dung + Maize stalks) $2.16 \times 10^9 \text{ CFU g}^{-1}$ to $2.03 \times 10^8 \text{ CFU g}^{-1}$ (Table 4.21).

Finally the viability was significantly more in T_5 (Cow dung + Fruit waste) $0.6 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $1.3 \times 10^6 \text{ CFU g}^{-1}$ at the end of tenth week and comparatively less viability was observed in T_4 (Cow dung + Maize stalks) $1.0 \times 10^9 \text{ CFU g}^{-1}$ on the first day to $1.0 \times 10^6 \text{ CFU g}^{-1}$ at the end of tenth week (Table 4.21).

4.2.3 Viability of the beneficial microorganisms inoculated individually into the sterilized biogas manure samples.

4.2.3.1 Population of *Rhizobium* after the enrichment.

The biogas manure samples were sterilized by autoclaving at 121⁰C. There was no microbial population in the sterilized biogas manure samples.

The enrichment of the biogas manures was done by adding Yeast Extract Mannitol(YEM) broth (3.1×10^9 CFU ml⁻¹) (Table 4.16).

The population of *Rhizobium* on the first day was significantly more in T₆ (Cow dung alone) 3.0×10^9 CFU g⁻¹, compared to T₁ (Cow dung + Press mud) 2.6×10^9 CFU g⁻¹, T₂ (Cow dung + Poultry litter) 1.6×10^9 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.0×10^9 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 0.8×10^9 CFU g⁻¹ and less in T₃ (Cow dung + Kitchen waste) 0.6×10^9 CFU g⁻¹ (Table 4.22).

There was an increase in the population of *Rhizobium* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₅ (Cow dung + Fruit waste) 1.3×10^9 CFU g⁻¹ (0.8×10^9 CFU g⁻¹ on the first day to 2.1×10^9 CFU g⁻¹ at the end of fourth week) and less in T₄ (Cow dung + Maize stalks) 0.6×10^9 CFU g⁻¹ (1.0×10^9 CFU g⁻¹ on the first day to 1.6×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after the fourth week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 3.6×10^9 CFU g⁻¹ at the end of fourth week to 1.6×10^9 CFU g⁻¹ at the end of sixth week and less reduction was observed in T₃ (Cow dung + Kitchen waste) 1.6×10^9 CFU g⁻¹ to 0.4×10^9 CFU g⁻¹ and also in T₅ (Cow dung + Fruit waste) 2.1×10^9 CFU g⁻¹ to 1.0×10^9 CFU g⁻¹ (Table 4.22).

Finally the viability was significantly more in T₃ (Cow dung + Kitchen waste) from 0.6×10^9 CFU g⁻¹ on the first day to 2.0×10^7 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₆ (Cow dung alone) 3.0×10^9 CFU g⁻¹ on the first day to 3.4×10^7 CFU g⁻¹ at the end of tenth week (Table 4.22).

4.2.3.2 Population of *Pseudomonas* after the enrichment.

The enrichment of the biogas manures was done by adding Kings B broth (3.0×10^9 CFU ml⁻¹) (Table 4.16).

The population of *Pseudomonas* on the first day was significantly more in T₆ (Cow dung alone) 2.8×10^9 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) CFU g⁻¹, T₁ (Cow dung + Press mud) CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.2×10^9 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.0×10^9 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 0.8×10^9 CFU g⁻¹ (Table 4.23).

There was an increase in the population of *Pseudomonas* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₃ (Cow dung + Kitchen waste) 1.5×10^9 CFU g⁻¹ (1.0×10^9 CFU g⁻¹ on the first day to 2.5×10^9 CFU g⁻¹ at the end of fourth week) and less in T₆ (Cow dung alone) 1.0×10^9 CFU g⁻¹ (2.8×10^9 CFU g⁻¹ on the first day to 3.8×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after the end of fourth week and significantly more reduction in the population was observed in T₂ (Cow dung + Poultry litter) 3.4×10^9 CFU g⁻¹ at the end of fourth week to 2.1×10^9 CFU g⁻¹ at the end of sixth week and less reduction was observed in T₆ (Cow dung alone) 3.8×10^9 CFU g⁻¹ to 3.1×10^9 CFU g⁻¹ (Table 4.23).

Finally the viability was significantly more in T₃ (Cow dung + Kitchen waste) from 1.0×10^9 CFU g⁻¹ on the first day to 1.6×10^7 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₄ (Cow dung + Maize stalks) 1.2×10^9 CFU g⁻¹ on the first day to 1.1×10^6 CFU g⁻¹ at the end of tenth week (Table 4.23).

4.2.3.3 Population of *Azotobacter* after the enrichment.

The enrichment of the biogas manures was done by adding *Azotobacter* glucose broth (2.6×10^9 CFU ml⁻¹) (Table 4.16).

The population of *Azotobacter* on the first day was significantly more in T₆ (Cow dung alone) 2.4×10^9 CFU g⁻¹, compared to T₁ (Cow dung + Press mud) 2.0×10^9 CFU g⁻¹, T₂ (Cow dung + Poultry litter) 1.8×10^9 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.4×10^9 CFU g⁻¹, T₅ (Cow dung + Fruit waste) 1.0×10^9 CFU g⁻¹ and less in T₄ (Cow dung + Maize stalks) 0.7×10^9 CFU g⁻¹ (Table 4.24).

There was an increase in the population of *Azotobacter* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₄ (Cow dung + Maize stalks) 2.3×10^9 CFU g⁻¹ (0.7×10^9 CFU g⁻¹ on the first day to 3.0×10^9 CFU g⁻¹ at the end of fourth week) and less in T₅ (Cow dung + Fruit waste) 1.3×10^9 CFU g⁻¹ (1.0×10^9 CFU g⁻¹ on the first day to 2.3×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after the end of fourth week and significantly more reduction in the population was observed in T₄ (Cow dung + Maize stalks) 3.0×10^9 CFU g⁻¹ at the end of fourth week to 1.56×10^8 CFU g⁻¹ at the end of sixth week and less reduction in T₅ (Cow dung + Fruit waste) 2.4×10^9 CFU g⁻¹ to 2.2×10^8 CFU g⁻¹ (Table 4.24).

Finally the viability was significantly more in T₂ (Cow dung + Poultry litter) 1.8×10^9 on the first day to 3.0×10^7 CFU g⁻¹ at the end of tenth week and comparatively

less viability was observed in T₃ (Cow dung + Kitchen waste) 1.4×10^9 CFU g⁻¹ on the first day to 2.2×10^7 CFU g⁻¹ at the end of tenth week (Table 4.24).

4.2.3.4 Population of *Azospirillum* after the enrichment.

The enrichment of the biogas manures was done by adding Potato infusion broth (2.6×10^9 CFU ml⁻¹) (Table 4.16).

The population of *Azospirillum* on the first day was significantly more in T₆ (Cow dung alone) 2.4×10^9 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 2.2×10^9 CFU g⁻¹, T₁ (Cow dung + Press mud) 2.0×10^9 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.2×10^9 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 0.6×10^9 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 0.2×10^9 CFU g⁻¹ (Table 4.25).

There was an increase in the population of *Azospirillum* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₄ (Cow dung + Maize stalks) 1.4×10^9 CFU g⁻¹ (0.6×10^9 CFU g⁻¹ on the first day to 2.0×10^9 CFU g⁻¹ at the end of fourth week) and less in T₂ (Cow dung + Poultry litter) 1.1×10^9 CFU g⁻¹ (2.2×10^9 CFU g⁻¹ on the first day to 3.3×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after the fourth week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 3.2×10^9 CFU g⁻¹ at the end of fourth week to 2.0×10^8 CFU g⁻¹ at the end of sixth week and less reduction in T₅ (Cow dung + Fruit waste) 1.6×10^9 CFU g⁻¹ to 1.0×10^8 CFU g⁻¹ (Table 4.25).

Finally the viability was significantly more in T₁ (Cow dung + Press mud) 2.0×10^9 CFU g⁻¹ on the first day to 3.1×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₂ (Cow dung + Poultry litter) 2.2×10^9 CFU g⁻¹ on the first day to 2.8×10^6 CFU g⁻¹ at the end of tenth week (Table 4.25).

The viability appears to be good upto the end of fourth week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium*, *Pseudomonas* and *Azospirillum* where as for *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manures.

4.2.4 Viability of the beneficial microorganisms in the unsterilized biogas manure samples after the enrichment with consortia of beneficial microorganisms.

4.2.4.1 Population of *Rhizobium* after the enrichment

The population of *Rhizobium* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 3.4×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 3.2×10^8 CFU g⁻¹, T₁ (Cow dung + Press

mud) 3.0×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 3.0×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.6×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 2.4×10^8 CFU g⁻¹ (Table 4.26).

There was an increase in the population of *Rhizobium* in all the six treatments till third week. The rate of multiplication was significantly more in T₆ (Cow dung alone) 1.4×10^8 CFU g⁻¹ (3.4×10^8 CFU g⁻¹ in the first day to 4.8×10^8 CFU g⁻¹ at the end of third week) and less in T₅ (Cow dung + Fruit waste) 0.6×10^8 CFU g⁻¹ (2.4×10^8 CFU g⁻¹ in the first day to 3.0×10^8 CFU g⁻¹ at the end of third week). The decrease in population was observed at the end of third week and significantly more reduction in the population was observed in T₆ (Cow dung alone) 4.8×10^8 CFU g⁻¹ at the end of third week to 3.2×10^8 CFU g⁻¹ at the end of fourth week and less reduction in T₃ (Cow dung + Kitchen waste) 3.8×10^8 CFU g⁻¹ to 3.2×10^8 CFU g⁻¹ and also in T₄ (Cow dung + Maize stalks) 3.2×10^8 CFU g⁻¹ to 2.6×10^8 CFU g⁻¹ (Table 4.26).

Finally the viability was significantly more in T₅ (Cow dung + Fruit waste) 2.4×10^8 CFU g⁻¹ on the first day to 3.0×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₃ (Cow dung + Kitchen waste) 2.6×10^8 CFU g⁻¹ on the first day to 2.6×10^6 CFU g⁻¹ at the end of tenth week (Table 4.26).

4.2.4.2 Population of *Pseudomonas* after the enrichment.

The population of *Pseudomonas* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 3.8×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 3.4×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 3.2×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 2.8×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.4×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 2.0×10^8 CFU g⁻¹ (Table 4.27).

There was an increase in the population of *Pseudomonas* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₂ (Cow dung + Poultry litter) 2.9×10^9 CFU g⁻¹ (3.4×10^8 CFU g⁻¹ on the first day to 3.3×10^9 CFU g⁻¹ at the end of fourth week) and less in T₄ (Cow dung + Maize stalks) 1.7×10^9 CFU g⁻¹ (2.4×10^8 CFU g⁻¹ on the first day to 2.0×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after fourth week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 3.0×10^9 CFU g⁻¹ at the end of fourth week to 2.8×10^8 CFU g⁻¹ in the sixth week and less reduction in T₆ (Cow dung alone) 3.6×10^9 CFU g⁻¹ to 4.2×10^8 CFU g⁻¹ (Table 4.27).

Finally the viability was significantly more in T₅ (Cow dung + Fruit waste) 2.0×10^8 CFU g⁻¹ on the first day to 1.8×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₆ (Cow dung alone) 3.8×10^8 CFU g⁻¹ on the first day to 2.8×10^6 CFU g⁻¹ at the end of tenth week (Table 4.27).

4.2.4.3 Population of *Azotobacter* after the enrichment.

The population of *Azotobacter* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 3.2×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 3.0×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 2.8×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 2.6×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.2×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 2.0×10^8 CFU g⁻¹ (Table 4.28).

There was an increase in the population of *Azotobacter* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₄ (Cow dung + Maize stalks) 1.9×10^9 CFU g⁻¹ (2.2×10^8 CFU g⁻¹ on the first day to 2.2×10^9 CFU g⁻¹ at the end of third week) and less in T₅ (Cow dung + Fruit waste) 1.6×10^9 CFU g⁻¹ (2.0×10^8 CFU g⁻¹ in the first day to 1.8×10^9 CFU g⁻¹ at the end of third week). The decrease in population was observed after third week and significantly more reduction in the population was observed in T₄ (Cow dung + Maize stalks) 2.2×10^9 CFU g⁻¹ at the end of third week to 2.2×10^8 CFU g⁻¹ at the end of fourth week and less reduction in T₁ (Cow dung + Press mud) 2.8×10^9 CFU g⁻¹ to 3.0×10^8 CFU g⁻¹ (Table 4.28).

Finally the viability was significantly more in T₆ (Cow dung alone) 3.2×10^8 CFU g⁻¹ on the first day to 3.2×10^6 CFU g⁻¹ at the end of tenth week and also in T₄ (Cow dung + Maize stalks) 2.2×10^8 CFU g⁻¹ on the first day to 2.2×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₅ (Cow dung + Fruit waste) 2.0×10^8 CFU g⁻¹ on the first day to 1.6×10^6 CFU g⁻¹ at the end of tenth week (Table 4.28).

4.2.4.4 Population of *Azospirillum* after the enrichment.

The population of *Azospirillum* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 3.7×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 3.6×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 3.4×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 3.0×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.6×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 2.2×10^8 CFU g⁻¹ (Table 4.29).

There was an increase in the population of *Azospirillum* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₆ (Cow dung

alone) 2.8×10^9 CFU g⁻¹ (3.7×10^8 CFU g⁻¹ on the first day to 3.2×10^9 CFU g⁻¹ at the end of third week) and less in T₁ (Cow dung + Press mud) 2.2×10^9 CFU g⁻¹ (3.4×10^8 CFU g⁻¹ on the first day to 2.6×10^9 CFU g⁻¹ at the end of third week). The decrease in population was observed after the third week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 2.6×10^9 CFU g⁻¹ at the end of third week to 3.0×10^9 CFU g⁻¹ at the end of fourth week and less reduction in T₆ (Cow dung alone) 3.2×10^9 CFU g⁻¹ at the end of third week to 3.4×10^8 CFU g⁻¹ at the end of fourth week (Table 4.29).

Finally the viability was significantly more in T₆ (Cow dung alone) 3.7×10^8 CFU g⁻¹ on the first day to 3.4×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₅ (Cow dung + Fruit waste) 2.2×10^8 CFU g⁻¹ on the first day to 1.6×10^6 CFU g⁻¹ at the end of tenth week (Table 4.29).

4.2.5 Viability of the beneficial microorganisms in the sterilized biogas manure samples after the enrichment with consortia of beneficial microorganisms.

4.2.5.1 Population of *Rhizobium* after the enrichment.

The population of *Rhizobium* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 2.8×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 2.6×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 2.4×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 2×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.6×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 1.2×10^8 CFU g⁻¹ (Table 4.30).

There was an increase in the population of *Rhizobium* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₃ (Cow dung + Kitchen waste) 2.8×10^9 (2.0×10^8 CFU g⁻¹ on the first day to 3.0×10^9 CFU g⁻¹ at the end of third week). The decrease in population was observed after third week and significantly more reduction in the population was observed in T₂ (Cow dung + Poultry litter) 3.6×10^9 CFU g⁻¹ at the end of third week to 3.0×10^8 CFU g⁻¹ at the end of fourth week and also in T₃ (Cow dung + Kitchen waste) 3×10^9 CFU g⁻¹ at the end of third week to 2.4×10^8 CFU g⁻¹ at the end of fourth week and less reduction in T₁ (Cow dung + Press mud) 3.4×10^9 CFU g⁻¹ to 3.2×10^8 CFU g⁻¹ (Table 4.30).

Finally the viability was significantly more in T₁ (Cow dung + Press mud) 2.4×10^8 CFU g⁻¹ on the first day to 3.4×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₅ (Cow dung + Fruit waste) 1.2×10^8 CFU g⁻¹ on the first day to 1.4×10^6 CFU g⁻¹ at the end of tenth week (Table 4.30).

4.2.5.2 Population of *Pseudomonas* after the enrichment.

The population of *Pseudomonas* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 3.4×10^8 CFU g⁻¹, compared to T₂ (Cow dung + Poultry litter) 3.2×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 3.0×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 3.0×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.6×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 2.4×10^8 CFU g⁻¹ (Table 4.31).

There was an increase in the population of *Pseudomonas* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₆ (Cow dung alone) 1.4×10^8 CFU g⁻¹ (3.4×10^8 CFU g⁻¹ on the first day to 4.8×10^8 CFU g⁻¹ at the end of third week) and less in T₅ (Cow dung + Fruit waste) 0.6×10^8 CFU g⁻¹ (2.4×10^8 CFU g⁻¹ on the first day to 3.0×10^8 CFU g⁻¹ at the end of third week). The decrease in population was observed after third week and significantly more reduction in the population was observed in T₆ (Cow dung alone) 4.8×10^8 CFU g⁻¹ at the end of third week to 3.2×10^8 CFU g⁻¹ at the end of fourth week and less reduction in T₃ (Cow dung + Kitchen waste) 3.8×10^8 CFU g⁻¹ at the end of third week to 3.2×10^8 CFU g⁻¹ at the end of fourth week and also in T₄ (Cow dung + Maize stalks) 3.2×10^8 CFU g⁻¹ to 2.6×10^8 CFU g⁻¹ (Table 4.31).

Finally the viability was significantly more in T₅ (Cow dung + Fruit waste) 2.4×10^8 CFU g⁻¹ in the first day to 3.0×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₃ (Cow dung + Kitchen waste) 3.0×10^8 CFU g⁻¹ in the first day to 2.6×10^6 CFU g⁻¹ at the end of tenth week (Table 4.31).

4.2.5.3 Population of *Azotobacter* after the enrichment.

The population of *Azotobacter* on the first day after the enrichment with beneficial microorganisms was significantly more in T₂ (Cow dung + Poultry litter) 3.2×10^8 CFU g⁻¹, compared to T₆ (Cow dung alone) 3.0×10^8 CFU g⁻¹, T₁ (Cow dung + Press mud) 2.4×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 2.1×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 2.0×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 1.8×10^8 CFU g⁻¹ (Table 4.32).

There was an increase in the population of *Azotobacter* in all the six treatments until the end of fourth week. The rate of multiplication was significantly more in T₃ (Cow dung + Kitchen waste) 1.9×10^9 CFU g⁻¹ (2.2×10^8 CFU g⁻¹ on the first day to 2.2×10^9 CFU g⁻¹ at the end of fourth week) and less in T₅ (Cow dung + Fruit waste) 1.4×10^9 CFU g⁻¹ (1.8×10^8 CFU g⁻¹ on the first day to 1.6×10^9 CFU g⁻¹ at the end of fourth week). The decrease in population was observed after the fourth week and

significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 2.4×10^9 CFU g⁻¹ at the end of fourth week to 3.2×10^8 CFU g⁻¹ at the end of sixth week and less reduction in T₄ (Cow dung + Maize stalks) 1.8×10^9 CFU g⁻¹ to 2.2×10^8 CFU g⁻¹ and also in T₅ (Cow dung + Fruit waste) 1.6×10^9 CFU g⁻¹ to 2.0×10^8 CFU g⁻¹ (Table 4.32).

Finally the viability was significantly more in T₁ (Cow dung + Press mud) 2.4×10^8 CFU g⁻¹ on the first day to 3.1×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₂ (Cow dung + Poultry litter) 3.2×10^8 CFU g⁻¹ on the first day to 3.1×10^6 CFU g⁻¹ at the end of tenth week (Table 4.32).

4.2.5.4 Population of *Azospirillum* after the enrichment

The population of *Azospirillum* on the first day after the enrichment with beneficial microorganisms was significantly more in T₆ (Cow dung alone) 2.8×10^8 CFU g⁻¹, compared to T₁ (Cow dung + Press mud) 2.4×10^8 CFU g⁻¹, T₂ (Cow dung + Poultry litter) 2.0×10^8 CFU g⁻¹, T₃ (Cow dung + Kitchen waste) 1.8×10^8 CFU g⁻¹, T₄ (Cow dung + Maize stalks) 1.4×10^8 CFU g⁻¹ and less in T₅ (Cow dung + Fruit waste) 1.2×10^8 CFU g⁻¹ (Table 4.33).

There was an increase in the population of *Azospirillum* in all the six treatments until the end of third week. The rate of multiplication was significantly more in T₂ (Cow dung + Poultry litter) 1.2×10^8 CFU g⁻¹ (2.0×10^8 CFU g⁻¹ on the first day to 3.2×10^8 CFU g⁻¹ at the end of third week) and less in T₁ (Cow dung + Press mud) 0.6×10^8 (2.4×10^8 CFU g⁻¹ on the first day to 3.0×10^8 CFU g⁻¹ at the end of third week). The decrease in population was observed after the third week and significantly more reduction in the population was observed in T₁ (Cow dung + Press mud) 3.0×10^8 CFU g⁻¹ at the end of third week to 2.4×10^8 CFU g⁻¹ at the end of fourth week and also in T₄ (Cow dung + Maize stalks) and in T₅ (Cow dung + Fruit waste) and less reduction in T₂ (Cow dung + Poultry litter) 3.2×10^8 CFU g⁻¹ at the end of third week to 3.0×10^8 CFU g⁻¹ at the end of fourth week and in T₃ (Cow dung + Kitchen waste) (Table 4.33).

Finally the viability was significantly more in T₂ (Cow dung + Poultry litter) 2.0×10^8 CFU g⁻¹ on the first day to 3.0×10^6 CFU g⁻¹ at the end of tenth week and comparatively less viability was observed in T₆ (Cow dung alone) 2.8×10^8 CFU g⁻¹ on the first day to 3.0×10^6 CFU g⁻¹ at the end of tenth week (Table 4.33).

The viability appears to be good upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium*, and *Azospirillum* where as for *Pseudomonas*, the viability was good upto the end of fourth week irrespective of treatments in unsterilized biogas manures and upto the end

of third week in sterilized manure samples. For *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments in unsterilized biogas manures and upto the end of fourth week in sterilized biogas manures.

Shruthi *et al.* (2014) carried work on the survivability of *Bacillus megatherium* in different carrier materials for improved shelf life of biofertilizer. The microbial population was estimated once in 30 days upto 240 days of storage (13.3×10^7 CFU g⁻¹ at 30 days and 2×10^7 CFU g⁻¹ at 240 days). Maximum viability of the organism was observed in press mud based biofertilizer because it was a rich source of nutrients especially carbon which favours the microorganisms survivability, compared to vermicompost, then lignite and was lowest in cocopeat.

Karmegam *et al.* (2012) conducted research on the enrichment of biogas slurry vermicompost with *Azotobacter chroococcum* and *Bacillus megatherium*. The inoculum level of *Azotobacter chroococcum* and *Bacillus megatherium* at rate of 35ml per 175g of vermibed substrate is sufficient to maintain 1×10^7 viable cells up to 160 days after harvesting of vermicompost. The inoculum of biofertilizer organisms in vermibed on 30th day showed increased viability rate and hence, the optimized inoculation of 35 ml of inoculum per 175 g of substrate on 30th day of vermicomposting is helpful for maintenance of sufficient viable population for more than five months in the enriched vermicompost.

Chapter V

SUMMARY AND CONCLUSIONS

The experiment entitled **“BIOGAS PRODUCTION FROM AGRICULTURAL WASTES AND FEASIBILITY STUDY TO ENRICH BIOGAS MANURES WITH BENEFICIAL MICROORGANISMS”** was conducted during 2013-14 at Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar, ANGRAU, Hyderabad.

Cow dung along with other agricultural wastes (press mud, poultry litter, kitchen wastes, maize stalks and fruit wastes) were used for the biogas production in lab scale. Along with the estimation of biogas production different parameters like Total Solids (TS) per cent, Total Volatile Solids (TVS) per cent, Volatile Fatty Acids (VFA), pH, Nitrogen (N) per cent, Phosphorous (P) per cent, Potassium (K) per cent, Organic Carbon per cent, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Electrical Conductivity (E.C) and methane percentage was estimated by using standard procedures.

Biogas production unit was established in the lab scale with six treatments and three replications each and with 750 g substrate and 1500 ml water in three litres glass bottles.

Significantly highest gas production was observed in T₁ (Cow dung + Press mud) 9903.31 ml compared to T₆ (Cow dung alone) 8103.31 ml, T₂ (Cow dung + Poultry litter) 6079.98 ml, T₃ (Cow dung + Kitchen waste) 4066.63 ml, T₅ (Cow dung + Fruit waste) 3373.32 ml and less in T₄ (Cow dung + Maize stalks) 3099.97 ml.

Total solids (TS) per cent in the substrate was significantly more in T₆ (Cow dung alone) 18.93 per cent and comparatively less in T₃ (Cow dung + Kitchen waste) 11.13 per cent. By the end of the process, the degradation of total solids (TS) per cent was recorded and it was significantly more in T₆ (Cow dung alone) 57.16 per cent and less in T₅ (Cow dung + Fruit waste) 32.55 per cent.

Total volatile solids (TVS) per cent in the substrates was significantly more in T₅ (Cow dung + Fruit waste) 89.34 per cent and comparatively less in T₁ (Cow dung + Press mud) 72.40 per cent. By the end of the process, the degradation of total volatile solids (TVS) per cent was recorded and it was significantly more in T₆ (Cow dung alone) 37.41 per cent and less in T₅ (Cow dung + Fruit waste) 14.32 per cent.

In the substrate VFA concentration was significantly more in T₃ (Cow dung + Kitchen waste) 1.19 g l⁻¹ and comparatively less in T₅ (Cow dung + Fruit waste) 0.38 g l⁻¹. At the end of the process the VFA concentration was significantly more in T₄ (Cow dung + Maize stalks) 2.27g l⁻¹ and less in T₁ (Cow dung + Press mud) 1.98 g l⁻¹.

pH in the substrate was significantly more in T₁ (Cow dung + Press mud) 7.66 and comparatively less in T₃ (Cow dung + Kitchen waste) 5.77. By the end of the process it was significantly more in T₆ (Cow dung alone) 6.82 and less in T₅ (Cow dung + Fruit waste) 3.67. The EC in the substrate was estimated and it was significantly more in T₁ (Cow dung + Press mud) 5.42 dsm⁻¹ and less in T₆ (Cow dung alone) 2.2 dsm⁻¹. At the end of the process the EC was estimated and it was more in T₁ (Cow dung + Press mud) 2.80 dsm⁻¹ and comparatively less in T₄ (Cow dung + Maize stalks) 1.23 dsm⁻¹.

Initially the N per cent increased gradually in all the six treatments until the end of third week and then decreased gradually. The N per cent in the substrates was significantly more in T₂ (Cow dung + Poultry litter) 2.62 per cent and less in T₄ (Cow dung + Maize stalks) 1.34 per cent. Significantly less reduction in N percent was observed in T₁ (Cow dung + Press mud) 14.29 percent. The P per cent in the substrates was significantly more in T₁ (Cow dung + Press mud) 1.87 percent and less in T₄ (Cow dung + Maize stalks) 0.70 per cent. By the end of the process P per cent in the digesters was significantly reduced and less reduction was observed in T₁ (Cow dung+ press mud) 17.12 per cent. The K per cent in the substrates was significantly more in T₂ (Cow dung + Poultry litter) 1.25 per cent and less in T₄ (Cow dung + Maize stalks) 0.50 per cent. At the end of the process K per cent in the digester increased and the increase was significantly more in T₁ (Cow dung + Press mud) 40.95 per cent.

The organic carbon per cent in the substrates was significantly more in T₆ (Cow dung alone) 52.15 per cent and less in T₄ (Cow dung + Maize stalks) 36.18 per cent. The reduction in organic carbon per cent was observed in all the treatments at the end of the process. The reduction was significantly more in T₁ (Cow dung + Press mud) 40.66 per cent and less in T₆ (Cow dung alone) 36.51 per cent. Biological oxygen demand in the substrates was estimated and it was significantly more in T₆ (Cow dung alone) 167.18 mg l⁻¹ and less in T₅ (Cow dung + Fruit waste) 79.58 mg l⁻¹. At the end of the process the reduction in BOD was observed and significantly more reduction was in T₅ (Cow dung + Fruit waste) 58.74 per cent and less reduction in T₄ (Cow dung + Maize stalks) 27.68 per cent. Chemical oxygen demand in the substrate was estimated and it was significantly more in T₆ (Cow dung alone) 148.61g l⁻¹ and less in T₂ (Cow dung + Poultry litter) 109.55g l⁻¹. At the end of the process decrease in COD was observed in

all the 6 treatments and more decrease was in T₆ (Cow dung alone) 52.15 and less reduction in T₄ (Cow dung + Maize stalks) 18.77 per cent.

Methane per cent was highest in the gas evolved from T₄ (Cow dung + Maize stalks) 52.51 per cent and lowest in T₃ (Cow dung + Kitchen waste) 47.95 per cent.

The beneficial micro organisms used in the study for enrichment of dried biogas spent slurry (biogas manures) were *Rhizobium*, *Pseudomonas*, *Azotobacter* and *Azospirillum*. The organisms were inoculated individually and as consortia in to the biogas manure samples of all the six treatments and the viability was monitored. The slurry samples from all the six treatments and three replications were dried in trays under the sun until the moisture was 50 per cent. The dried manure samples were divided into two and one part was sterilized and the other was used as the same i.e. unsterilized. The population of beneficial micro organisms in the dried manures was estimated and the population of *Pseudomonas* ($46.0 \times 10^3 \text{CFU g}^{-1}$) and *Azospirillum* ($24.0 \times 10^3 \text{CFU g}^{-1}$) was more in T₆ (Cow dung alone), the population of *Rhizobium* was more in T₁ (Cow dung + Press mud) $46.0 \times 10^3 \text{CFU g}^{-1}$ and the population of *Azotobacter* was more in T₂ (Cow dung + Poultry litter) and T₆ (Cow dung alone) $42.0 \times 10^3 \text{CFU g}^{-1}$.

In the unsterilized manure samples enriched with beneficial micro organisms individually the viability of *Rhizobium* was significantly more in T₅ (Cow dung + Fruit waste), for *Pseudomonas* viability was significantly more in T₃ (Cow dung + Kitchen waste) for *Azotobacter* viability was significantly more in T₆ (Cow dung alone) and for *Azospirillum* viability was significantly more in T₅ (Cow dung + Fruit waste). In the sterilized manure samples the viability of *Rhizobium* was significantly more in T₃ (Cow dung + Kitchen waste), for *Pseudomonas* viability was significantly more in T₃ (Cow dung + Kitchen waste), for *Azotobacter* viability was significantly more in T₂ (Cow dung + Poultry litter) and for *Azospirillum* viability was significantly more in T₁ (Cow dung + Press mud).

The viability appears to be good upto the end of fourth week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium*, *Pseudomonas* and *Azospirillum* where as for *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manures.

In the unsterilized manure samples enriched with beneficial micro organisms altogether the viability of *Rhizobium* was significantly more in T₅ (Cow dung + Fruit waste), for *Pseudomonas* the viability was significantly more in T₅ (Cow dung + Fruit

waste), for *Azotobacter* viability was significantly more in T₆ (Cow dung alone) and for *Azospirillum* viability was significantly more in T₆ (Cow dung alone). In the sterilized manure samples the viability of *Rhizobium* was significantly more in T₁ (Cow dung + Press mud) for *Pseudomonas* viability was significantly more in T₅ (Cow dung + Fruit waste), for *Azotobacter* viability was significantly more in T₁ (Cow dung + Press mud) and for *Azospirillum* viability was significantly more in T₂ (Cow dung + Poultry litter).

The viability appears to be good upto the end of third week irrespective of treatments and also in unsterilized and sterilized biogas manure samples for *Rhizobium*, and *Azospirillum* where as for *Pseudomonas*, the viability was good upto the end of fourth week irrespective of treatments in unsterilized biogas manures and upto the end of third week in sterilized manure samples. For *Azotobacter* the viability appears to be good only upto the end of third week irrespective of treatments in unsterilized biogas manures and upto the end of fourth week in sterilized biogas manures.

The combination of cow dung + Press mud (T₁) was found to be the best in biogas production. However, all the combinations were also proved to be better for the biogas production. Though K per cent increased in all the slurry samples, reduction in N per cent and P per cent was observed but less reduction was observed in T₁ (Cow dung + Press mud). Hence it can be concluded that T₁ (Cow dung + Press mud) was comparatively better in N, P and K composition while all other combinations were also good and can be used as biogas manures.

The beneficial microorganisms used in the present study showed viability and the population of added beneficial microorganisms increase was observed in different biogas manures samples indicating that the biogas manure samples from different substrates support the beneficial microorganisms population for atleast 3-4 weeks duration.

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APPENDIX 1

Composition of different growth media/reagents/indicators used

1. Azotobacter agar (Glucose)

Dipotassium phosphate	1.00 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.20 g
Ferrous Sulphate	0.005 g
Soil extract	5.00 g
Glucose	10.00 g
Agar	20 g
Distilled water	1000 ml
pH	7.4 – 7.8

2. Azotobacter Broth (Glucose)

Dipotassium phosphate	1.00 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.20 g
Ferrous Sulphate	0.005 g
Soil extract	5.00 g
Glucose	10.00 g
Distilled water	1000 ml
pH	7.4 – 7.8

3. Potato Infusion Agar

Potato infusion form	200 g
Peptic digest of animal tissue	10.00 g
Beef extract	5.00 g
Dextrose	10.00 g
Sodium chloride	15.00 g
Agar	15.00g
Distilled water	1000 ml
pH	6.6 – 7.0

4. Kings B agar medium (king *et al.*, 1954)

Peptone	16.0 g
Dipotassium hydrogen phosphate	1.6 g
Magnesium sulphate	1.6 g
Glycerol	10.0 g
Agar	18.00 g
Distilled water	1000 ml

5. Yeast Extract Mannitol Congored Agar

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Congo red	0.025 g
Agar	20.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

6. Yeast Extract Mannitol Broth

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Calcium carbonate	1.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

7. Nutrient Agar

Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml

pH	6.8 – 7.2
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8. Gram Stain Solutions

a) Crystal violet solution

Crystal violet	10.0 g
Ammonium oxalate	4.0 g
Ethanol	100 ml
Distilled water	1000 ml

b) Gram's Iodine solution

Iodine	1.0 g
Potassium iodide	2.0 g
Ethanol	25 ml
Distilled water	100 ml

c) Counter stain

2.5% safranin in ethanol	10 ml
Distilled water	100 ml

d) Ethyl alcohol (Decolouriser)

Ethanol	95 ml
Distilled water	5 ml

APPENDIX 2

Reagents used for chemical analysis of substrates used for biogas production and biogas slurry samples

1. pH

Standard buffer solution: Prepare buffer solution of pH 4.0, 7.0 and 9.2 in pure water, or of other pH value in the acidic and alkaline range. Alternatively, dissolve one commercially available buffer tablet in distilled water and make the volume of 1000 ml. Prepare a fresh buffer solution after every few days as these solutions do not keep for long. A 0.05 M solution of AR grade potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$, mol. wt. 204.22) gives a pH of 4.00 at 25°C and this can also be used as a standard buffer. For this prepare a stock solution of 0.25 M by dissolving 51.04 gm of the pure dry salt in warm water and making volume to 1000 ml. three to four drops of toluene are added to prevent the growth of mould. A 10 ml portion of this solution diluted to 50 ml with water gives 0.05 M solution. Similarly, a solution containing 19.45 ml of 0.2 M Na_2HPO_4 and 0.55 ml of 0.1M citric acid gives a buffer of pH 8.0.

2. Electrical Conductivity

0.01 N Potassium chloride solution: Dry a small quantity of AR grade potassium chloride at 60 °C for 2 h. Weigh 0.7456 g of it and dissolve in freshly prepared distilled water and make to one litre. This solution gives an electrical conductivity of 1411.8×10^{-3} i.e. 1.41 dSm⁻¹ at 25 °C.

3. Organic Carbon

Reagents:-

- 1) 1N Potassium dichromate: Dissolve 49.04g of AR grade $\text{K}_2\text{Cr}_2\text{O}_7$ in about 500 ml of distilled water and make the volume to one litre.
- 2) Conc. Sulphuric acid
- 3) 0.5N Ferrous ammonium sulphate: Dissolve 196g of ferrous ammonium sulphate in distilled water, add 20ml of conc. H_2SO_4 and make up volume to one litre. The ferrous ammonium sulphate should be from fresh lot and in light green colour. Yellowing of the salt indicates its oxidation.
- 4) Diphenylamine indicator: Dissolve 0.5g of the dye in a mixture of 20ml of distilled water and 100 ml of conc. H_2SO_4 .
- 5) Orthophosphoric acid (85%) or sodium fluoride.

4. Nitrogen

Reagents:-

1. Sulphuric acid : Salicylic acid mixture – Dissolve one gram of salicylic acid in 30mL of Con. H_2SO_4 .
2. 0.1 N H_2SO_4 – Dissolve approximately 2.8 mL of Conc. H_2SO_4 in one litre of distilled water in volumetric flask and standardize against 0.1 N sodium carbonate or 0.1 N borax.
3. 40% NaOH: Dissolve 2.5g of sodium hydroxide pellet in water and make to one litre.
4. 4% Boric acid: Dissolve 20g of boric acid powder in warm water by stirring and dilute to one litre.
5. Mixed indicator: Dissolve 0.5g of Bromocresol green + 0.1 g of methyl red in 100ml of 95% ethanol and adjust to pH 4.5.
6. CuSO_4
7. K_2SO_4
8. $\text{Na}_2\text{S}_2\text{O}_3$
9. Zinc pieces

5. Phosphorus

Reagents:-

- 1) 100 mg l^{-1} P solution: Weigh 0.439 g of AR grade KH_2PO_4 dried in oven at 60°C for 1h in a one litre beaker, add about 500 ml of distilled water and dissolve. Add 25ml of approx. 7N H_2SO_4 and make up the vol. to one litre.
- 2) 2 mg l^{-1} P working solution: Dilute a suitable volume of 100 mg l^{-1} P solution by 50 times to get 2 mg l^{-1} P solution.
- 3) Tri acid mixture: Nitric acid: Sulphuric acid: Perchloric acid at 9:2:1
- 4) Barton's reagent

6. Potassium

Reagents:-

- 1) Standard K solution: prepare 1g l^{-1} solution by dissolving 1.908 g of dried (in oven) KCl salt per solution. Dilute suitable vol. of this solution to get

100 ml of working standards containing 10, 15, 20, 25, 30 and 40 mg K L⁻¹

¹. The working standards should be prepared in the medium of extraction.

7. Biological Oxygen Demand

Reagents:-

- 1) Manganous sulphate solution: Dissolve 100g of manganous sulphate in 200mL of previously boiled distilled water and filter the solution.
- 2) Alkaline potassium iodide solution: Weigh 50g of potassium iodide and 100g of potassium hydroxide. Dissolve the chemicals in 200mL of previously boiled distilled water.
- 3) Sodium thiosulphate solution (0.025N): Take 6.205g of sodium thiosulphate, dissolve it in previously boiled distilled water and make up the volume to 1 L. Add a pallet of sodium hydroxide as a preservative. Keep the solution in coloured bottle.
- 4) Starch indicator: Dissolve 1g of starch in 100mL of warm distilled water and add few drops of toluene or formaldehyde as preservative.
- 5) BOD-free water: Pass the deionized glass distilled water through a column of activated carbon and redistill it.
- 6) Phosphate buffer solution: Dissolve 42.5g of potassium dihydrogen phosphate in 700ml BOD free water and add 8.8g NaOH. Adjust the pH 7.2. Add 2g ammonium sulphate and dilute to 1 litre with BOD free water.
- 7) Magnesium sulphate solution: Dissolve 82.5g of magnesium sulphate in BOD free distilled water to prepare 1 L of solution.
- 8) Calcium chloride solution: Dissolve 27.5g of anhydrous calcium chloride in BOD free distilled water to prepare 1 L solution.
- 9) Ferric chloride solution: Dissolve 0.25g of ferric chloride in 1 L of BOD free distilled water.
- 10) Sulphuric acid (1N): Add 2.8 mL of concentrated sulphuric acid to 100ml of BOD free distilled water.
- 11) Sodium hydroxide solution (1N): Add 4g of sodium hydroxide in BOD free distilled water and make the volume 100 mL.

12) Allyl thiourea solution: Dissolve 500mg of allylthiourea in distilled water and make the volume 1L.

1) Chemical Oxygen Demand

Reagents:-

- 1) Potassium dichromate solution(0.25N): Dissolve 12.259g of AR grade potassium dichromate previously dried at 103⁰C in distilled water. Add about 120mg sulphuric acid to this and dilute to 1L.
- 2) Dry powder of silver sulphate
- 3) Dry powder of mercuric sulphate
- 4) Concentrated sulphuric acid
- 5) Ferrion indicator solution: Dissolve 0.695g of ferrous sulphate and 1.485g of 1, 10-phenonthroline in distilled water to make 100mL of indicator solution.
- 6) Standard ferrous ammonium sulphate solution (0.25N): Dissolve 98g of ferrous ammonium sulphate in distilled water add 20mL of sulphuric acid, cool and dilute to 1L further by adding distilled water.



Plate 3: Muffle furnace with crucibles for estimating Total Volatile Solids in the slurry samples



Plate 4: Flame photometer for estimation of K per cent in the slurry samples and hot plate with slurry samples kept for digestion.

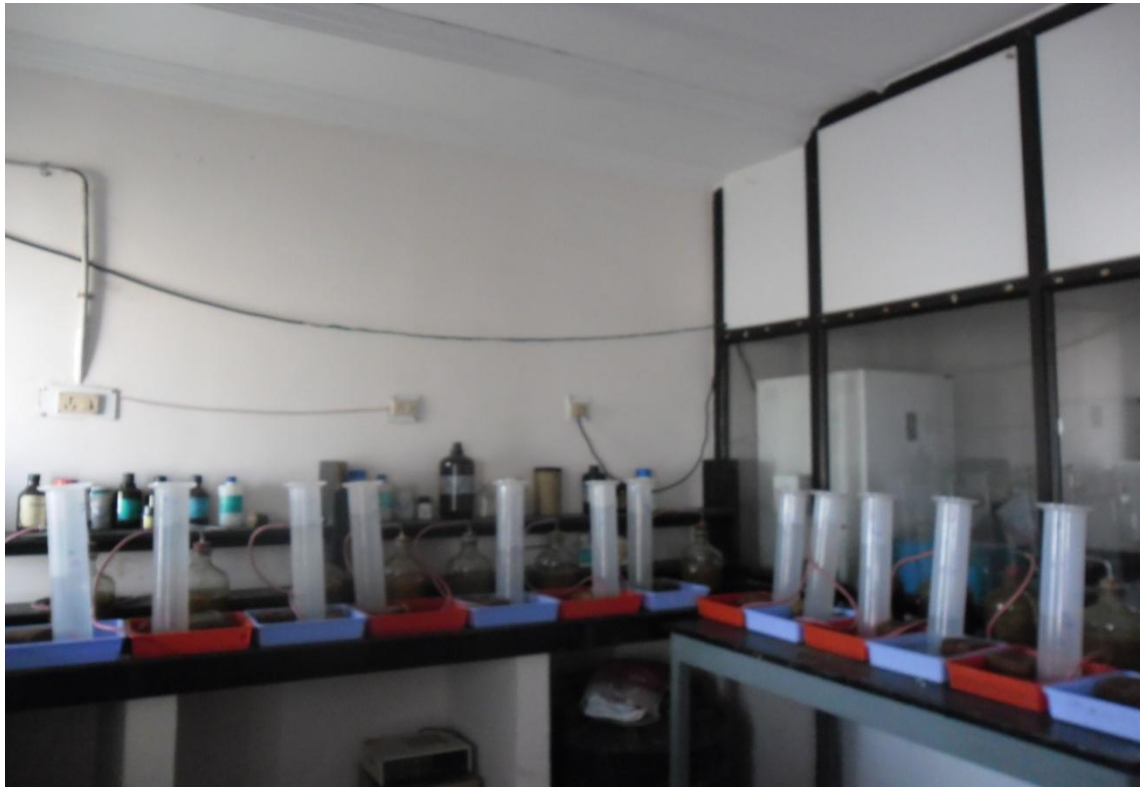


Plate 1: Biogas unit set in the lab scale



Plate 2: Maize stalks used for biogas production



Plate 5: Closed reflux unit apparatus for estimation of COD in the slurry samples



Plate 6: BOD estimation in the slurry samples



Plate 7: Gas chromatographic estimation of methane percentage in the biogas

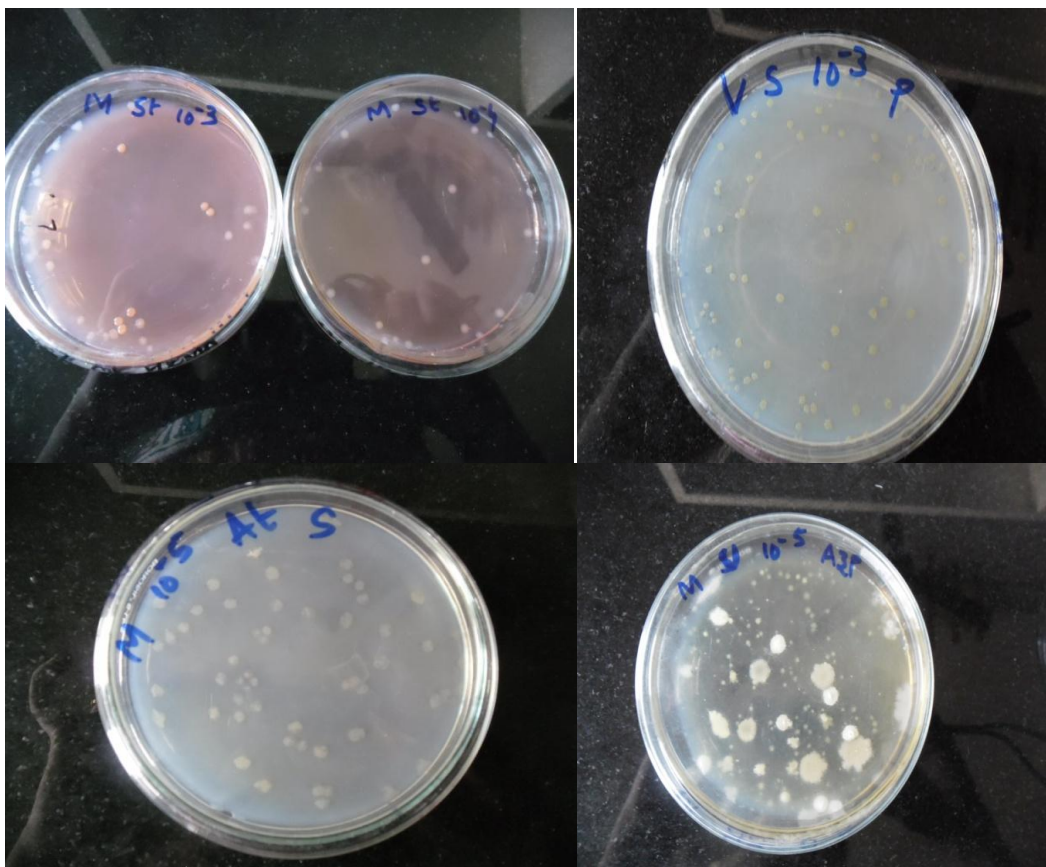


Plate 8: Beneficial microorganisms (*Rhizobium*, *Pseudomonas*, *Azotobacter*, *Azospirillum*) isolated from the enriched biogas manure samples.